UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: January 24th, 2018

SUBJECT: **Afidopyropen**: Report of the Cancer Assessment Review Committee

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Health Effects Division (7509P)

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The Cancer Assessment Review Committee met on October 18 and November 1, 2017 to evaluate the cancer classification of afidopyropen in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

AFIDOPYROPEN

PC CODE 026200

January 24th, 2018

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

The Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met twice to evaluate the carcinogenic potential of afidopyropen. On October 18, 2017, the CARC evaluated the adequacy of dosing and the study findings in carcinogenicity studies in rats and mice, as well as the results from the genotoxicity studies. On November 1, 2017, the CARC reconvened to evaluate the Registrant submitted mechanistic studies and proposal to support a mode-of-action (MOA) for uterine tumors in female rats, and to determine the cancer classification for afidopyropen. This report summarizes the findings and conclusions from both meetings.

Rats

In two combined chronic/carcinogenicity studies in rats, afidopyropen was administered to 50 male and 50 female Fisher rats per group in the diet at dose levels of 0, 100, 300, or 1000 ppm (low dose study; 0/0, 4/5, 13/16, or 43/51 mg/kg bw/day males/females, respectively) [MRID 49688983] or 0, 1000, or 3000 ppm (high dose study; 0/0, 42/51, 128/147 mg/kg bw/day males/females, respectively) [MRID 49688984] for 2 years. In addition, a battery of mechanistic studies was submitted to support a proposed MOA for uterine tumor formation in female rats.

Because there were no statistically significant survival disparities between the dose groups of the low and high dose rat studies and the studies were conducted in the same strain and under the same testing conditions, the studies were combined for a more comprehensive analysis. The following considerations were discussed:

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the adrenal tumors in males did not display a dose response, were not statistically significant (Fisher's Exact Test or Exact Trend Test), and there were no corresponding non-neoplastic lesions at any dose level. **The CARC concluded that the adrenal tumors were not treatment-related at any dose tested.**

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the combined lung tumors (adenomas and carcinomas) in males did not reach statistical significance (Fisher's Exact Test or Exact Trend Test), and no corresponding non-neoplastic lesions were noted at any dose level. **The CARC concluded that the lung tumors were not treatment-related at any dose tested.**

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the benign liver tumors (adenomas) in males did reach statistical significance ($p \leq 0.05$ for both Fisher's Exact Test and Exact Trend Test) and was outside of the historical control range and above the historical control mean at 3000 ppm. However, there was a weak dose response, no evidence of a progression to malignant tumors (carcinomas), was observed and the only noteworthy non-neoplastic finding was altered foci (predominantly

"slight" in the degree of severity). **The CARC concluded that the liver adenomas in male rats were treatment-related at 3000 ppm (128 mg/kg bw/day).**

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the uterine tumors in females displayed a dose response, reached statistical significance at 1000 and 3000 ppm ($p \leq 0.01$ for both Fisher's Exact Test and Exact Trend Test) for both adenocarcinomas and combined adenocarcinomas and adenomas, were outside the historical control range (at 3000 ppm), were above the historical control mean (at 1000 and 3000 ppm), and displayed uterine hyperplasia (increased incidence at 1000 and 3000 ppm). **The CARC concluded that the uterine tumors were treatment-related at 1000 and 3000 ppm (51 and 147 mg/kg bw/day, respectively).**

- The CARC concluded that dosing was adequate and not excessive for the combined low and high dose carcinogenicity studies in the rat based on effects in the mandibular lymph node and the liver, and an increase in the number of pituitary cysts in males. In females, adverse histopathology was noted in the liver, pituitary, and eye lens at ≥1000 ppm, with additional adverse effects observed at 3000 ppm.

The Registrant submitted mechanistic studies and a proposal to support a MOA for uterine tumors in female rats. The Registrant proposed a dopamine enhancement mode of action *via* agonism of the dopamine receptor, leading to decreased serum prolactin levels, and through a cascade of key events, uterine tumors. **The CARC concluded that the submitted data do not adequately support the proposed MOA** based on the following considerations:

- Key Event #1: The CARC concluded that there is no direct evidence from the Registrant submitted studies that afidopyropen is an agonist of the dopamine receptor. Even though multiple *in silico* and *in vitro* binding assay results were presented, the overall weight-of-evidence conclusion still did not support agonism of the dopamine receptor for either parent or any metabolites tested.

- Key Event # 2: The CARC concluded that there is not sufficient evidence across all submitted studies to support a decrease in serum prolactin levels. There is limited evidence of lower prolactin concentrations at the high dose in one study, but this effect was not consistently observed or present to the same extent as the positive control chemical (bromocriptine) and was only reported at a higher dose level (361 - 404 mg/kg/day) than the tumorigenic dose level (50 mg/kg/day). In addition, prolactin concentrations were *higher* in the 92-day study at 1000 ppm, where a tumorigenic effect was seen in the carcinogenicity study. The only evidence of decreased prolactin at 1000 ppm was limited to the "metestrus group" on day 24, but such stage-specific effects would potentially be confounded by altered estrous cyclicity (noted in other studies).

- Key Event #3: The CARC concluded that there is no direct evidence or data from the Registrant submitted studies that afidopyropen decreases corpus luteum support leading to decreased production of progesterone and a resulting estrogen dominance.

- Key Event #4: The CARC concluded that there is evidence of altered cyclicity, but the effects occur inconsistently across studies; do not correspond well with those of bromocriptine, the positive control agent; and do not support delayed senescence at the tumorigenic dose (1000 ppm). There was a decrease in mammary duct dilation at 3000 ppm; however, (a) no mammary gland effects seen at 12 months in the high-dose study or 24 months at the tumorigenic dose (1000 ppm) in either the low-dose or high-dose rat studies; (b) when the results were combined from both studies, the dose response diminishes; and (c) this effect is not necessarily specific to altered reproductive senescence in aged rats.

- Key Event #5: The CARC concluded that there is not sufficient evidence for an increase in endometrial hyperplasia at 1000 ppm when looked at across both the low dose and high dose rat carcinogenicity studies. There is evidence to support an increase in endometrial hyperplasia at the 3000 ppm dose at 24 months. When the data from both studies are combined, the dose response diminishes, and there was no increase in endometrial hyperplasia seen at 12 months in the high-dose study. The CARC concluded there was weak evidence of endometrial hyperplasia at 3000 ppm.

- Key Event #6*:* The CARC concluded that there is evidence to support the formation of uterine tumors and determined the tumors to be treatment-related at 1000 and 3000 ppm.

Mice

In a mouse carcinogenicity study, afidopyropen was administered to 52 ICR mice/sex/dose *via* the diet at dose levels of 0, 120, 700 and 4000 ppm (0/0, 13.3/12.9, 78.7/75.8, 445/333 mg/kg bw/day ♂/♀) for 78 weeks [MRID 49688985]. In the high dose group in females, the dose was changed to 3000 ppm at week 24, and then to 2000 ppm at week 44 due to death or moribundity.

- There were no treatment-related tumors in the mouse carcinogenicity study.

- Dosing was considered adequate and not excessive in the mouse carcinogenicity study based on a decrease in absolute body weight in females, clinical signs, increases in hematological blood parameters, spleen and ovary weights, an increase in pale colored liver.

Mutagenicity

- There is no concern for mutagenicity as determined from a battery of genotoxicity assays.

Structural Activity Relationships

- There are no structurally similar chemicals to inform structural activity relationships (SAR) for afidopyropen.

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified afidopyropen as "***Suggestive Evidence of Carcinogenic Potential***" based on uterine tumors in female rats. There is insufficient evidence to support the proposed uterine tumor MOA in female rats. There is no concern for mutagenicity.

The *Final Guidelines for Carcinogen Risk Assessment* (March, 2005) were consulted regarding the benign liver tumors in male rats. The *Guidelines* state:

- *"Benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis. There is a range of possibilities for their overall significance."*
- *"[O]bservation of a benign tumor response alone may have no significant health hazard implications when other sources of evidence show no suggestion of carcinogenicity."*
- *"[I]n assessing findings from animal studies, a greater proportion of malignancy is weighed more heavily than is a response with a greater proportion of benign tumors."*

In the case of afidopyropen, the incidence of liver adenomas in male rats was not significantly increased in either the low dose or high dose study alone; a statistically significant increase was observed at the highest dose tested (3000 ppm) only when the studies were combined. These tumors did not progress to malignancy in either the low or high dose rat carcinogenicity studies and were not considered treatment-related in the mouse carcinogenicity study (due to the small magnitude of malignant neoplasms and the relative comparison to historical controls). The only noteworthy non-neoplastic finding was altered cell foci of the liver. Neither hypertrophy or hyperplasia were noted in the low dose or high dose rat carcinogenicity study. While the CARC did consider the liver adenomas to be treatment-related, based on the weight-of-evidence and guidance noted above, the benign tumors were not considered a concern in the overall cancer classification. The classification of "*Suggestive Evidence of Carcinogenic Potential*" is based on the uterine tumors in female rats (one sex/one species).

Quantification of human cancer risk is not required. The chronic Reference Dose (RfD) will adequately account for all chronic toxicity, including carcinogenicity, which could result from exposure to afidopyropen

I. INTRODUCTION

The Cancer Assessment Review Committee (CARC) met on October 18 and November 1, 2017, to evaluate the carcinogenic potential of afidopyropen and determine a cancer classification in accordance with the *EPA's Guidelines for Carcinogen Risk Assessment* (US EPA, 2005). The findings from the rodent carcinogenicity studies and genotoxicity studies, and the adequacy of dosing were evaluated at the October 18, 2017 meeting. The data submitted to support the Registrant's proposed mode-of-action (MOA) for uterine tumors in female rats were considered in a separate meeting on November 1, 2017.

II. BACKGROUND

Afidopyropen is a new active ingredient (ai) being proposed for use as an insecticide on various agricultural crops, vegetables for transplant, and ornamentals (including those in greenhouses and nurseries). The four end-use products included in this proposal are all liquid formulations, containing either 4.89% ai (0.42 lb ai/gal) or 9.78% ai (0.83 lb ai/gal). Broadcast foliar applications are being proposed via aerial, groundboom, airblast, chemigation and/or handheld equipment, depending on the crop/use site, at maximum rates ranging from 0.010 to 0.046 lb ai/A (or 0.00045 lb ai/gal). Tolerances are being proposed for residues of afidopyropen on various commodities ranging from 0.01 ppm (tree nut crop group, soybean seed, tuberous and corm vegetable subgroup) to 5 ppm (leafy vegetable subgroup).

The proposed pesticidal MOA is gate disruption of transient receptor potential vanilloid (TRPV) channel complexes in chordotonal stretch receptor organs of insects. These channel complexes are critical for hearing, sensory modality, balance, proprioception and kinesthesia, and disruption ultimately causes alterations in feeding and other behaviors. The mammalian MOA is not known at this time.

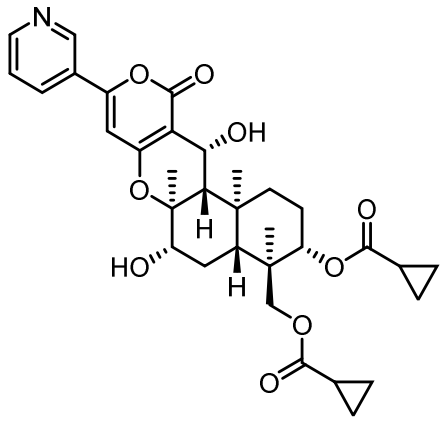


Figure 1. Structure of Afidopyropen

III. EVALUATION OF CARCINOGENICITY STUDIES

Two combined chronic/carcinogenicity studies with afidopyropen were conducted in rats. In the

first (low dose) study (MRID 49688983) afidopyropen was administered to rats at 0, 100, 300 and 1000 ppm. The Dose Adequacy Review Team (DART) of the Health Effects Division (HED) of the US EPA reviewed the dose levels while the study was ongoing and concluded that an additional dose of 3000 ppm was required to meet the maximum tolerated dose (MTD) requirements. The agency also requested that the 1000 ppm dose be repeated to bridge the results to the ongoing rat cancer study that was being conducted at dose levels of 100, 300 and 1000 ppm (November 18, 2010; TXR# 0055537). Therefore, a second (high dose) combined chronic/carcinogenicity study (MRID 49688984) was conducted in rats at dose levels of 0, 1000, and 3000 ppm for 2 years.

In a mouse carcinogenicity study, afidopyropen was administered to ICR mice *via* the diet at dose levels of 0, 120, 700 and 4000 ppm for 78 weeks (MRID 49688985). In the high dose female group, the dose was changed to 3000 ppm at week 24, and then to 2000 ppm at week 44 due to death or moribundity.

Throughout this document the low dose carcinogenicity data will be discussed first followed by the high dose carcinogenicity data. Because there were no statistically significant survival disparities between the dose groups of the low and high dose rat studies, and these studies were conducted in the same strain of rat at the same testing laboratory and same relative time period, these studies have been combined for a more comprehensive analysis, and will be discussed following the individual study data. Finally, the mouse carcinogenicity study (MRID 49688985) data will be discussed.

1. Combined chronic/carcinogenicity study with afidopyropen in F344/DuCrlCrlj rats (low dose)

Reference: Yamashita, R (2014). Carcinogenicity Study of ME5343 Technical in Rats. Nisseiken Co. Ltd. Laboratory report number: C-32. Applicant Report Number: 2014/8000287. MRID 49688983.

A. Experimental Design

In a carcinogenicity study, ME5343 technical (95.74% a.i.) was administered to 50 Fischer rats/sex/dose via the diet at dose levels of 0, 100, 300, or 1000 ppm (0/0, 4.4/5.3, 12.9/15.5, or 42.7/50.8 mg/kg bw/day ♂/♀) for 2 years.

B. Survival Analysis

There were no statistically significant survival disparities among the dose groups in male or female rats of the low dose study (**Table 1** and **Table 2**).

Table 1. Afidopyropen – Fischer Low Dose Rat Study
(MRID No. 49688983)

Male Mortality Rates+ and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	**1-26**	**27-52**	**53-78**	**79-104[f]**	**Total**
0	0/50	0/50	1/50	10/49	11/50 (22)
100	0/50	0/50	3/50	7/47	10/50 (20)
300	0/50	0/50	0/50	14/50	14/50 (28)
1000	0/50	0/50	2/50	8/48	10/50 (20)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
Number in parenthesis indicates percent mortality rate.
[f] Final sacrifice at week 104.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Afidopyropen – Fischer Low Dose Rat Study
(MRID No. 49688983)

Female Mortality Rates+ and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	**1-26**	**27-52**	**53-78**	**79-104[f]**	**Total**
0	0/50	0/50	2/50	11/48	13/50 (26)
100	0/50	0/50	6/50	6/44	12/50 (24)
300	0/50	0/50	0/50	8/50	8/50 (16)
1000	0/50	0/50	1/50	10/49	11/50 (22)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
Number in parenthesis indicates percent mortality rate.
[f] Final sacrifice at week 104.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Discussion of Tumor Data

Historical control data were not available from the facility conducting the study (Nisseiken Co. Tokyo, Japan). However, upon request, the Registrant provided historical control data from BoZo Research Center, Japan, which obtained F344/DuCrlCrlj rats from the same breeding center (Atsugi Breeding Center, Charles River, Japan, Inc.) as used by the conducting laboratory for the afidopyropen carcinogenicity studies. Details on study year, route of administration, vehicle, and number of animals from the historical control experiments are provided below.

Sex:	Male						
Study ID:	#18	#19	#20	#21-1	#21-2	**Mean (%)**	*Range (%)*
Necropsy at the End of Administration (Year):	2010	2010	2011	2015	2015		
Study weeks:	104	104	104	104	104		
Route of administration:	PO	PO	PO	PC	PC		
Vehicle*:	DW	TC-5	MC	Placebo	-		
Number examined:	55	55	55	55	55	**275**	

Sex:	Female						
Study ID:	#18	#19	#20	#21-1	#21-2	**Mean (%)**	*Range (%)*
Necropsy at the End of Administration (Year):	2010	2010	2011	2015	2015		
Study weeks:	104	104	104	104	104		
Route of administration:	PO	PO	PO	PC	PC		
Vehicle*:	DW	TC-5	MC	Placebo	-		
Number examined:	55	55	55	55	55	**275**	

*: DW: drinking water; TC-5: hypromellose; MC: methylcellulose; –: not treated

Male rats had a statistically significant trend for adrenal pheochromocytomas at $p < 0.01$ and for adrenal pheochromocytomas and/or malignant pheochromocytomas combined at $p < 0.05$. There were no statistically significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend (**Table 3a**). In addition, the incidence of benign pheochromocytomas at 1000 ppm (20%) was outside of the historical control range (5-16%) and above the mean (11.3%) (**Table 3b**).

Table 3a. Afidopyropen – Fischer Low Dose Rat Study
(MRID No. 49688983)

Male Adrenal Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	**100**	**300**	**1000**
Pheochromocytomas (%)	4/50 (8)	2/50 (4)	4/50 (8)	10[a]/50 (20)
P =	0.0063**	0.8978	0.6425	0.0739
Malignant Pheochromocytomas (%)	1[b]/50 (2)	0/50 (0)	1/50 (2)	0/50 (0)
P =	0.3744	1.0000	0.7525	1.0000
Combined (%)	5/50 (10)	2/50 (4)	5/50 (10)	10/50 (20)
P =	0.0142*	0.9441	0.6297	0.1312

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a] First pheochromocytoma observed at week 63 in the 1000 ppm dose group.
[b] First malignant pheochromocytoma observed at week 84 in the control group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3b. Historical Control Data for Adrenal Tumors in F344/DuCrlCrlj Male Rats

Tumor Type	Mean (%)	Range (%)
Pheochromocytoma, benign	11.3	5 – 16
Pheochromocytoma, malignant	1.1	0 – 4
Pheochromocytoma, complex, malignant	0.7	0 – 4

No statistically significant trend or pairwise comparisons were noted for male liver tumors (**Table 4a**) or male lung tumors (**Table 5a**) (Fisher's Exact Test or Exact Test for Trend). No dose response was observed for liver tumors and the incidence of liver tumors at 1000 ppm was within the historical control range and below the historical control mean (**Table 4b**). No dose response was observed for lung adenomas or combined adenomas and/or carcinomas and the incidence of tumors was within historical control range for adenomas at 1000 ppm, but outside of historical control range for carcinomas at 1000 ppm (**Table 5b**). It should be noted that lung adenomas/carcinomas can be difficult to distinguish histopathologically, so caution is warranted when making conclusions based on small differences in incidence between adenomas and carcinomas.

Table 4a. Afidopyropen – Fischer Low Dose Rat Study
(MRID No. 49688983)

Male Liver Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	**100@**	**300@**	**1000**
Hepatocellular Adenomas (%)	1[a]/50 (2)	2[a]/31 (6)	0/31 (0)	1[a]/50 (2)
P =	0.3708	0.3252	1.0000	0.7525

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
#No hepatocellular carcinomas were observed.
@Not all animals were examined in the 100 and 300 ppm dose groups.
[a] First hepatocellular adenoma observed at the final sacrifice simultaneously in the control, 100 and 1000 ppm dose groups.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4b. Historical Control Data for Liver Tumors in F344/DuCrlCrlj Male Rats

Tumor Type	Mean (%)	Range (%)
Adenoma, hepatocellular	4.0	0 – 5

Table 5a. Afidopyropen – Fischer Low Dose Rat Study
(MRID No. 49688983)

Male Lung Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	**100#**	**300#**	**1000**
Bronchiolar Alveolar Adenomas (%)	5[a]/50 (10)	0/12 (0)	1[a]/15 (7)	3[a]/50 (6)
P =	0.3364	1.0000	0.8076	0.8657
Bronchiolar Alveolar Carcinomas (%)	0/50 (0)	0/12 (0)	0/15 (0)	2[b]/50 (4)
P =	0.1531	1.0000	1.0000	0.2475
Combined (%)	5/50 (10)	0/12 (0)	1/15 (7)	5/50 (10)
P =	0.3985	1.0000	0.8076	0.6297

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
#Not all animals were examined in the 100 and 300 ppm dose groups.
[a] First bronchiolar alveolar adenoma observed at the final sacrifice simultaneously in the control, 300 and 1000 ppm dose groups.
[b] First bronchiolar alveolar carcinoma observed at week 104 in the 1000 ppm dose group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5b. Historical Control Data for Lung (Bronchus) Tumors in F344/DuCrlCrlj Male Rats

Tumor Type	Mean (%)	Range (%)
Adenoma, bronchiolo-alveolar	3.6	2 – 7
Carcinoma, bronchiolo-alveolar	0.4	0 – 2

Female rats had statistically significant trends for uterine adenocarcinomas and adenomas and/or adenocarcinomas combined, both at $p < 0.01$. There were no statistically significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the tumors in female rats were based upon Fisher's Exact Test and the Exact Test for Trend (**Table 6a**). Adenomas at 300 and 1000 ppm were outside of the historical control range and above the mean; however, so were the concurrent controls. Adenocarcinomas were within the historical control range but above of the mean at 1000 ppm (**Table 6b**).

Table 6a. Afidopyropen – Fischer Low Dose Rat Study
(MRID No. 49688983)

Female Uterine Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	**100**	**300**	**1000**
Adenomas (%) P =	2[a]/50 (4) 0.2192	1/50 (2) 0.8788	2/50 (4) 0.6914	3/50 (6) 0.5000
Adenocarcinomas (%) P =	4[b]/50 (8) 0.0020**	1/50 (2) 0.9719	2/50 (4) 0.8978	10/50 (20) 0.0739
Combined (%) P =	6/50 (12) 0.0017**	2/50 (4) 0.9703	4/50 (8) 0.8411	13/50 (26) 0.0624

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a] First adenoma observed at week 89 in the control group.
[b] First carcinoma observed at week 85 in the control group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6b. Historical Control Data for Uterine Tumors in F344/DuCrlCrlj Female Rats

Tumor Type	Mean (%)	Range (%)
Adenoma, endometrial	0.4	0 – 2
Adenocarcinoma	9.1	0 – 22

D. Non-Neoplastic Lesions

In males, there was a slight increase in altered foci (basophilic; slight in degree of severity) and hepatic spongiosis (predominately slight in degree) of the liver at 1000 ppm. There was also a slight increase in osseous metaplasia (slight in degree) and fibrosis (slight in degree) of the lung at 1000 ppm (**Table 7**). However, statistical significance was not reached for any of the aforementioned histological findings.

Endometrial hyperplasia was noted in control and treated animals; however, a dose-response was not observed and it was not statistically different from concurrent control animals at any dose tested (**Table 8**).

Table 7. Afidopyropen – F344/DuCrlCrlj Rat Study (MRID No. 49688983)

Relevant Male Non-Neoplastic Lesions

ppm	0	100	300	1000
Males				
Liver: Foci, altered cell, basophilic, diffuse (slight)	1 / 50 (2%)	1 / 31 (3%)	0 / 31	3 / 50 (6%)
Liver: Foci, altered cell, basophilic, diffuse (moderate)	0 / 50	0 / 31	1 / 31 (3%)	0 / 50
Liver: Foci, altered cell, basophilic, diffuse (total)	1 / 50 (2%)	1 / 31 (3%)	1 / 31 (3%)	3 / 50 (6%)
Liver: Spongiosis, hepatis, (slight)	2 / 50 (4%)	1 / 31 (3%)	1 / 31 (3%)	6 / 50 (12%)
Liver: Spongiosis, hepatis, (moderate)	0 / 50	0 / 31	0 / 31	1 / 50 (2%)
Liver: Spongiosis, hepatis, (total)	2 / 50 (4%)	1 / 31 (3%)	1 / 31 (3%)	7 / 50 (14%)
Lung with bronchus: osseous metaplasia, intraalveola (slight)	1 / 50 (2%)	0 / 12	1 / 15 (7%)	3 / 50 (6%)
Lung with bronchus: fibrosis (slight)	0 / 50	0 / 12	0 / 15	1 / 50 (2%)

Data obtained from pages 113-157 in the study report (MRID 49688983)
Histopathology in all male rats (killed by design & killed by moribund and died during the study)
* Significantly different from control, $p<0.05$

Table 8. Afidopyropen – F344/DuCrlCrlj Rat Study (MRID No. 49688983)

Relevant Female Non-Neoplastic Lesions

ppm	0	100	300	1000
Females				
Uterus: endometrial hyperplasia (slight)	7 / 50 (14%)	8 / 50 (16%)	12 / 50 (24%)	9 / 50 (18%)
Uterus: endometrial hyperplasia (moderate)	2 / 50 (4%)	2 / 50 (4%)	1 / 50 (2%)	0 / 50
Uterus: endometrial hyperplasia (severe)	1 / 50 (2%)	0 / 50	0 / 50	1 / 50 (2%)
Uterus: endometrial hyperplasia (total)	10 / 50 (20%)	10 / 50 (20%)	13 / 50 (26%)	10 / 50 (20%)

Data obtained from pages 113-157 in the study report (MRID 49688983)
Histopathology in all female rats (killed by design & killed by moribund and died during the study)
* Significantly different from control, $p<0.05$

E. Adequacy of the Dosing for Assessment of Carcinogenicity

Dose selection for the low dose carcinogenicity study was based on results from a 90-day feeding study in rats (MRID 49688967). A Registrant established LOAEL of 1000 ppm was based on an increase in relative liver weight and a number of hematological and clinical chemistry parameters in both sexes, and histopathology findings in the liver and hearts of females. The NOAEL was set at 300 ppm.

The HED Dose Adequacy Review Team (DART) reviewed the dose levels in the ongoing 2-year rat chronic toxicity/carcinogenicity (low dose) study (MRID 49688983) and concluded that an additional dose of 3000 ppm was required to meet the agency's maximum tolerated dose (MTD) requirements. The agency also requested that the 1000 ppm dose be repeated to bridge the results to the ongoing rat cancer study that was being conducted at dose levels of 100, 300 and 1000 ppm (November 18, 2010; TXR# 0055537). This additional (high dose) study is discussed below (MRID 49688984).

Adequacy of dosing will be discussed in more detail for the combined data of the low and high dose rat carcinogenicity studies.

2. Combined chronic/carcinogenicity study with afidopyropen in F344/DuCrlCrlj rats (high dose study)

Reference: Oshima, A (2015). Carcinogenicity Study of BAS 440 I (Reg. No. 5599022, ME5343 technical) in Rats – Administration via the Diet. Nisseiken Co. Ltd. Laboratory report number: C-34. BASF Registration Document Number: 2014/1215781. MRID 49688984.

A. Experimental Design

In a carcinogenicity study, BAS 440 I (95.74% a.i.) was administered to 50 Fischer (F344/DuCrlCrlj) rats/sex/dose via the diet at dose levels of 0, 1000, or 3000 ppm (0/0, 41.6/50.4, or 128.2/146.9 mg/kg bw/day in ♂/♀) for 2 years.

B. Survival Analysis

There were no statistically significant survival disparities among the dose groups in male rats of the high dose study. Female rats of the high dose study had a marginally statistically significant ($p \leq 0.05$) increasing trend for mortality (**Table 9** and **Table 10**).

Table 9. Afidopyropen – Fischer High Dose Rat Study (MRID No. 49688984)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	**1-26**	**27-52**	**53-78**	**79-104[f]**	**Total**
0	0/50	0/50	1/50	9/49	10/50 (20)
1000	0/50	0/50	2/50	7/48	9/50 (18)
3000	0/50	0/50	1/50	6/49	7/50 (14)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f] Final sacrifice at week 104.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 10. Afidopyropen – Fischer High Dose Rat Study
(MRID No. 49688984)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	**1-26**	**27-52**	**53-78**	**79-104[f]**	**Total**
0	0/50	0/50	2/50	8/48	10/50 (20) *
1000	1/50	0/49	2/49	7/47	10/50 (20)
3000	0/50	0/50	2/50	16/48	18/50 (36)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f] Final sacrifice at week 104.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Discussion of Tumor Data

No statistical significance trend or pair-wise comparisons were noted for male adrenal tumors at any dose tested (**Table 11**). A dose response was not observed for pheochromocytomas or combined pheochromocytomas and/or malignant pheochromocytomas. Pheochromocytomas were within the historical control range and below the mean at 3000 ppm. Malignant adrenal tumors were slightly outside the historical control range at 3000 ppm; however, the concurrent controls were at the high end of the historical control range (**Table 3b**)

Table 11. Afidopyropen – Fischer High Dose Rat Study
(MRID No. 49688984)

Male Adrenal Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)		
	0	**1000**	**3000**
Pheochromocytomas (%) P =	8/50 (16) 0.0964	11[a]/50 (22) 0.3055	4/50 (8) 0.9394
Malignant Pheochromocytomas (%) P =	2[b]/50 (4) 0.3575	2[b]/50 (4) 0.6914	3[b]/50 (6) 0.5000
Combined (%) P =	10/50 (20) 0.1263	12[c]/50 (24) 0.4049	6[d]/50 (12) 0.9143

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a] First pheochromocytoma observed at week 90 in the 1000 ppm dose group.
[b] First malignant pheochromocytoma observed in the final sacrifice simultaneously in all dose groups.
[c] One animal in the 1000 ppm dose group had both a pheochromocytoma and malignant pheochromocytoma.
[d] One animal in the 3000 ppm dose group had both a pheochromocytoma and malignant pheochromocytoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

No statistical significance trend or pair-wise comparisons were noted for male liver tumors at any dose tested (**Table 12**). Hepatocellular adenomas were outside the historical control range and above the mean at 1000 and 3000 ppm (**Table 4b**).

Table 12. Afidopyropen – Fischer High Dose Rat Study
(MRID No. 49688984)

Male Liver Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)		
	0	**1000**	**3000**
Hepatocellular Adenomas# (%) P =	1[a]/50 (2) 0.06853	3[a]/50 (6) 0.30865	5/50 (10) 0.10220

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
#No hepatocellular carcinomas were observed
[a] First hepatocellular adenoma observed at the final sacrifice simultaneously in all dose groups.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Male rats had a statistically significant trend for bronchiolar-alveolar carcinomas at $p < 0.05$. There were no statistically significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the tumors in the high dose male rat study were based upon Fisher's Exact Test and the Exact Test for Trend (**Table 13**). Adenomas were within the historical control range at 1000 and 3000 ppm and carcinomas were outside of the historical control range and above the mean at 3000 ppm (**Table 5b**).

Table 13. Afidopyropen – Fischer High Dose Rat Study (MRID No. 49688984)

Male Lung Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)		
	0	**1000**	**3000**
Bronchiolar Alveolar Adenomas	4[a]/50	3[a]/50	3[a]/50
(%)	(8)	(6)	(6)
P =	0.4225	0.7820	0.7820
Bronchiolar Alveolar Carcinomas	0/50	0/50	3[b]/50
(%)	(0)	(0)	(6)
P =	0.0356*	1.0000	0.1212
Combined	4/50	3/50	6/50
(%)	(8)	(6)	(12)
P =	0.2314	0.7820	0.3703

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a] First bronchiolar alveolar adenoma observed at the final sacrifice simultaneously in the control, 300 and 1000 ppm dose groups.
[b] First bronchiolar alveolar carcinoma observed at the final sacrifice in the 3000 ppm dose group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Female rats had statistically significant trends, and statistically significant pair-wise comparisons of the 3000 dose group with the controls, for uterine adenocarcinomas and adenomas and/or adenocarcinomas combined, all at $p < 0.01$. There were statistically significant pair-wise comparisons of the 1000 ppm dose group with the controls for uterine adenocarcinomas at $p < 0.05$ and for uterine adenomas and/or adenocarcinomas combined at $p < 0.01$. The statistical analyses of the tumors in the high dose female rat study were based upon Peto's Prevalence Test (**Table 14**). A dose response was noted for adenomas, adenocarcinomas, and combined adenomas and/or adenocarcinomas. Adenomas were outside of the historical control range and above the mean at 1000 and 3000 pm. In addition, adenocarcinomas were outside the historical control range and above the mean at 3000 ppm (**Table 6b**).

Table 14. Afidopyropen – Fischer High Dose Rat Study
(MRID No. 49688984)

Female Uterine Tumor Rates+ and
Peto's Prevalence Test Results

	Dose (ppm)		
	0	**1000**	**3000**
Adenomas (%) P =	1/41 (2) 0.09670	3/41 (7) 0.15397	4[a]/36 (11) 0.08812
Adenocarcinomas (%) P =	0/49 (0) 0.00064**	5[b]/48 (10) 0.02546*	12/48 (25) 0.00018**
Combined (%) P =	1/49 (2) 0.00038**	8/48 (17) 0.00916**	15/48 (31) 0.00015**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a] First adenoma observed at week 101 in the 3000 ppm dose group.
[b] First carcinoma observed at week 74 in the 1000 ppm dose group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Non-Neoplastic Lesions

In males from the 3000 ppm group, the incidences of sinus dilatation (slight in degree) of the mandibular lymph node was increased ($p \leq 0.05$) when compared to concurrent controls (**Table 15).** Males in the 1000 and 3000 ppm group had a slight increase in fatty change of the adrenal cortex (slight in degree) as compared to controls; however, this was not statistically significant at any dose tested. Altered foci (clear cell type) of the liver were statistically significantly increased ($p \leq 0.05$) when compared to control at 3000 ppm. Also noted was a slight increase in the number of incidences of altered foci (eosinophilic); however, the increase was not statistically significant at any dose tested. There was a slight increase in liver degeneration (slight – moderate), spongiosis hepatis (slight), biliary cysts (slight) and hepatodiaphragmatic nodules at 1000 and 3000 ppm; however, statistical significance was not reached at any dose tested.

In females, the incidences of hyperplasia of the endometrium in the uterus were increased at 3000 ppm in females and found to be statistically significant ($p \leq 0.05$). A slight, not statistically significant, increase in uterine endometrial hyperplasia was also observed at 1000 ppm (**Table 16**).

Table 15. Afidopyropen – F344/DuCrlCrlj Rat Study (MRID No. 49688984)

Relevant Male Non-Neoplastic Lesions

ppm	0	1000	3000
Males			
Mandibular lymph node: Dilatation, sinus (slight)	11 / 49 (22%)	15 / 50 (30%)	*23 / 50 (46%)
Adrenal gland: fatty change, focal, cortex (slight)	17 / 50 (34%)	21 / 50 (42%)	23 / 50 (46%)
Liver: Foci, altered cell, clear cell (slight)	2 / 50 (4%)	4 / 50 (8%)	*10 / 50 (20%)
Liver: Foci, altered cell, clear cell (moderate)	1 / 50 (2%)	1 / 50 (2%)	2 / 50 (4%)
Liver: Foci, altered cell, clear cell (total)	3 / 50 (6%)	5 / 50 (10%)	*12 / 50 (24%)
Liver: Foci, altered cell, eosinophilic (slight)	36 / 50 (72%)	37 / 50 (74%)	37 / 50 (74%)
Liver: Foci, altered cell, eosinophilic (moderate)	3 / 50 (6%)	3 / 50 (6%)	4 / 50 (8%)
Liver: Foci, altered cell, eosinophilic (total)	39 / 50 (78%)	40 / 50 (80%)	41 / 50 (82%)
Liver: degeneration, centrilobule (slight)	0 / 50	1 / 50 (2%)	2 / 50 (4%)
Liver: degeneration, centrilobule (moderate)	0 / 50	2 / 50 (4%)	1 / 50 (2%)
Liver: degeneration, centrilobule (total)	0 / 50	3 / 50 (6%)	3 / 50 (6%)
Liver: Spongiosis hepatis (slight)	2 / 50 (4%)	3 / 50 (6%)	5 / 50 (10%)
Liver: biliary cyst (slight)	2 / 50 (4%)	4 / 50 (8%)	4 / 50 (8%)
Liver: hepatodiaphragmatic nodule	6 / 50 (12%)	7 / 50 (14%)	11 / 50 (22%)

Data obtained from pages 113-150 in the study report (MRID 49688984)
Histopathology in all male rats (killed by design & killed by moribund and died during the study)
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Incidence in percentage presented in parentheses.

Table 16. Afidopyropen – F344/DuCrlCrlj Rat Study (MRID No. 49688984)

Relevant Female Non-Neoplastic Lesions

ppm	0	1000	3000
Females			
Uterus: Hyperplasia, endometrium (slight)	7 / 50 (14%)	10 / 50 (20%)	13 / 50 (26%)
Uterus: Hyperplasia, endometrium (moderate)	0 / 50	0 / 50	1 / 50 (2%)
Uterus: Hyperplasia, endometrium (severe)	0 / 50	1 / 50 (2%)	2 / 50 (4%)
Uterus: Hyperplasia, endometrium (total)	7 / 50 (14%)	11 / 50 (22%)	*16 / 50 (32%)

Data obtained from pages 113-150 in the study report (MRID 49688984)
Histopathology in all female rats (killed by design & killed by moribund and died during the study)
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Incidence in percentage presented in parentheses.

E. Adequacy of the Dosing for Assessment of Carcinogenicity

Adequacy of dosing will be discussed in more detail for the combined data of the low and high dose rat carcinogenicity studies.

3. Combined statistics of low and high dose rat carcinogenicity studies (MRID 49688983 + MRID 49688984)

A. Survival Analyses

Because there were no statistically significant survival disparities between the dose groups of the low and high dose male rat studies, these studies have been combined for a more comprehensive analysis. No survival disparities were noted in males. Female rats of the high dose study had a marginally significant increasing trend for mortality; however, the significant trend for female mortality in the high dose study was borderline and was only statistically significant by one of 3 statistical tests and, therefore, it is still appropriate to combine the low and high dose studies. There was a statistically significant increasing trend for survival among the dose groups in the female rats of the combined studies (**Table 17** and **Table 18**).

Table 17. Afidopyropen – Combined Rat Studies
(MRID No. 49688983 and 49688984)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	**1-26**	**27-52**	**53-78**	**79-104[f]**	**Total**
0	0/100	0/100	2/100	19/98	21/100 (21)
100	0/50	0/50	3/50	7/47	10/50 (20)
300	0/50	0/50	0/50	14/50	14/50 (28)
1000	0/100	0/100	4/100	15/96	19/100 (19)
3000	0/50	0/50	1/50	6/49	7/50 (14)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f] Final sacrifice at week 104.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 18. Afidopyropen – Combined Rat Studies
(MRID No. 49688983 and 49688984)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	**1-26**	**27-52**	**53-78**	**79-104[f]**	**Total**
0	0/100	0/100	4/100	19/96	23/100 (23) *
100	0/50	0/50	6/50	6/44	12/50 (24)
300	0/50	0/50	0/50	8/50	8/50 (16)
1000	1/100	0/99	3/99	17/96	21/100 (21)
3000	0/50	0/50	2/50	16/48	18/50 (36)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f] Final sacrifice at week 104.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

B. Discussion of Tumor Data

No statistically significant trend or pair-wise comparisons were noted for male adrenal tumors at any dose tested (**Table 19**). A dose response was not observed for pheochromocytomas, malignant pheochromocytomas, or combined pheochromocytomas and/or malignant pheochromocytomas. Pheochromocytomas were within the historical control range and below the historical control mean at 3000 ppm. The malignant pheochromocytomas were slightly outside the historical control range and above the historical control mean at 3000 ppm (**Table 3b**). In addition, there is a lack of corresponding non-neoplastic lesions of the adrenal gland in both the low and high dose carcinogenicity studies in the rat.

The CARC concluded that the adrenal tumors in male rats are not treatment-related at any dose tested.

Table 19. Afidopyropen – Combined Rat Studies (MRID No. 49688983 and 49688984)

Male Adrenal Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)				
	0	**100**	**300**	**1000**	**3000**
Pheochromocytomas (%)	12/100 (12)	2/50 (4)	4/50 (8)	21[a]/100 (21)	4/50 (8)
P =	0.4715	0.9777	0.8486	0.0633	0.8486
Malignant Pheochromocytomas (%)	3[b]/100 (3)	0/50 (0)	1/50 (2)	2/100 (2)	3/50 (6)
P =	0.0814	1.0000	0.7525	0.8157	0.3173
Combined (%)	15/100 (15)	2/50 (4)	5/50 (10)	22[c]/100 (22)	6[c]/50 (12)
P =	0.4011	0.9932	0.8664	0.1372	0.7968

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a] First pheochromocytoma observed at week 63 in the 1000 ppm dose group.
[b] First malignant pheochromocytoma observed at week 84 in the control group.
[c] One animal in each of the 1000 and 3000 ppm dose groups had both a pheochromocytoma and a malignant pheochromocytoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Male rats of the combined studies had statistically significant trends for hepatocellular adenomas at $p < 0.05$. There were also statistically significant pair-wise comparisons of the 3000 ppm dose group for hepatocellular adenomas at $p < 0.05$. The statistical analyses of the tumors in the combined male rat studies were based upon Fisher's Exact Test and the Exact Test for Trend (**Table 20**). At 3000 ppm, adenomas were outside of the historical control range and above the historical control mean (**Table 4b**). However, there was a weak dose response, no malignant tumors were observed (carcinomas), and the only noteworthy non-neoplastic plastic finding was altered foci (predominantly slight in degree).

The CARC concluded that the liver adenomas (benign) were treatment-related at 3000 ppm.

Table 20. Afidopyropen – Combined Rat Studies
(MRID No. 49688983 and 49688984)

Male Liver Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)				
	0	**100@**	**300@**	**1000**	**3000**
Hepatocellular Adenomas# (%)	2[a]/100 (2)	2[a]/31 (6)	0/31 (0)	4[a]/100 (4)	5[a]/50 (10)
P =	0.01855*	0.23750	1.0000	0.34136	0.04140*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
#No hepatocellular carcinomas were observed.
@Not all animals examined in the 100 and 300 ppm dose groups
[a] First hepatocellular adenoma observed at the final sacrifice simultaneously in the control, 100, 1000 and 3000 ppm dose groups.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Male rats of the combined studies had statistically significant trends for bronchiolar-alveolar carcinomas at $p < 0.05$. There were also statistically significant pair-wise comparisons of the 3000 ppm dose group with the controls for bronchiolar-alveolar carcinomas at $p < 0.05$. There were no statistically significant trends or pair-wise comparisons for bronchiolar-alveolar adenomas alone or combined bronchiolar-alveolar adenomas and carcinomas. The statistical analyses of the tumors in the combined male rat studies were based upon Fisher's Exact Test and the Exact Test for Trend (**Table 21**). There were no non-neoplastic lesions noted at any dose level tested to support a treatment-related effect. In addition, it should be noted that distinguishing between adenomas/carcinomas histopathologically can be very difficult and may account for the slight increase in carcinomas.

The CARC concluded that the lung tumors were not treatment-related at any dose tested.

Table 21. Afidopyropen – Combined Rat Studies (MRID No. 49688983 and 49688984)

Male Lung Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)				
	0	**100**	**300**	**1000**	**3000**
Bronchiolar Alveolar Adenomas (%)	9[a]/100 (9)	0/12 (0)	1[a]/15 (7)	6[a]/100 (6)	3[a]/50 (6)
P =	0.3269	1.0000	0.7678	0.8586	0.8299
Bronchiolar Alveolar Carcinomas (%)	0/100 (0)	0/12 (0)	0/15 (0)	2[b]/100 (2)	3[b]/50 (6)
P =	0.0116*	1.0000	1.0000	0.2487	0.0356*
Combined (%)	9/100 (9)	0/12 (0)	1/15 (7)	8/100 (8)	6/50 (12)
P =	0.2082	1.0000	0.7678	0.6934	0.3777

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
#Not all animals were examined in the 100 and 300 ppm dose groups.
[a] First bronchiolar alveolar adenoma observed at the final sacrifice simultaneously in the control, 300, 1000 and 3000 ppm dose groups.
[b] First bronchiolar alveolar carcinoma observed at the final sacrifice simultaneously in the 1000 and 3000 ppm dose groups.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Female rats of the combined studies had statistically significant trends, and statistically significant pair-wise comparisons of the 1000 and 3000 ppm dose groups with the controls, for uterine adenocarcinomas and adenomas and/or adenocarcinomas combined, all at $p < 0.01$. A dose response was also noted for adenomas, adenocarcinomas, and combined adenomas and/or adenocarcinomas. The statistical analyses of the tumors in the combined female rat studies were based upon Peto's Prevalence Test (**Table 22**). Adenomas were outside of the historical control range and above the historical control mean at 300, 1000 and 3000 ppm and adenocarcinomas were outside of the historical control range and above the historical control mean at 3000 ppm (**Table 6b**). In addition, increased incidence of uterine hyperplasia was also observed at 3000 ppm which supports the observed tumors.

The CARC concluded that the uterine tumors were treatment-related at 1000 and 3000 ppm.

Table 22. Afidopyropen – Combined Rat Studies (MRID No. 49688983 and 49688984)

Female Uterine Tumor Rates+ and Peto's Prevalence Test Results

	Dose (ppm)				
	0	**100**	**300**	**1000**	**3000**
Adenomas (%)	3[a]/89 (3)	1/43 (2)	2/50 (4)	6/89 (7)	4/44 (9)
P =	0.05323	0.58978	0.42556	0.13754	0.13412
Adenocarcinomas (%)	4/97 (4)	1/47 (2)	2/50 (4)	15[b]/97 (15)	12/48 (25)
P =	0.00003**	0.79798	0.53960	0.00661**	0.00078**
Combined (%)	7/97 (7)	2/47 (4)	4/50 (8)	21/97 (22)	15[c]/48 (31)
P =	0.00002**	0.77894	0.46787	0.00274**	0.00132**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor
[a] First adenoma observed at week 89 in the control group.
[b] First carcinoma observed at week 74 in the 1000 ppm dose group.
[c] One animal in the 3000 ppm dose group had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Adequacy of the Dosing for Assessment of Carcinogenicity

The CARC concluded that dosing was adequate and not excessive for the combined low and high dose carcinogenicity studies in the rat. In males from the 3000 ppm group, adverse histopathology was noted in the mandibular lymph node and the liver, and an increase in the number of pituitary cysts was observed in the gross pathology findings.

In females, adverse histopathology was noted in the liver, pituitary (gross finding), and eye lens at ≥1000 ppm. At 3000 ppm, there was a decrease in final absolute body weight (↓13%) and an increase in the number of female deaths. Also at 3000 ppm, adverse histopathology was noted in the kidney and uterus.

The Registrant claims that nonlinear kinetics, saturation of elimination, occurred at doses ≥15 mg/kg/bw. This argument is discussed in detail (Section 5b of the Mode of Action Section). ***CARC concluded that saturation of elimination has not been adequately demonstrated***.

4. Carcinogenicity study with afidopyropen in ICR mice

Reference: Takahashi, N (2012). Carcinogenicity Study of ME5343 Technical in Mice. The Institute of Environmental Toxicology (Japan). Laboratory report number: IET 09-0027. Study report date: 23-February-2012. BASF Registration Document Number: 2012/8000283. MRID 49688985.

A. Experimental Design

In a mouse carcinogenicity study, ME5343 Technical (95.74 % a.i.) was administered to 52 ICR mice/sex/dose via the diet at dose levels of 0, 120, 700 and 4000 ppm (0/0, 13.3/12.9, 78.7/75.8, 445/333 mg/kg bw/day ♂/♀) for 78 weeks. In the high dose group in females, the dose was changed to 3000 ppm at week 24, and then to 2000 ppm at week 44 due to death or moribundity.

B. Survival Analysis

In the 4000 ppm group, a number of females were killed in extremis or found dead after 14 weeks of treatment. The cumulative death rate in females was 4/52 at week 21, and the value was statistically significantly different when compared to the control group (0/52 in the control females). The statistical significance was recorded until week 25.

The dose was reduced to 3000 ppm at week 24 in females. Furthermore, two females were killed in extremis at week 41, thereby reaching the cumulative death rate 6/52 (vs 1/52 in the control females). The dose was further reduced to 2000 ppm at week 44 and throughout the remainder of the study to prevent increase of mortality. The mortality rate became comparable to that of the control, and no significant difference was noted in the final mortality at the termination of treatment of the group (**Table 23**).

Table 23. Afidopyropen – Mouse Carcinogenicity Study (MRID No. 49688985)

Summary of cumulative mortality

ppm	**0 (n=52)**	**120 (n=52)**	**700 (n=52)**	**4000 (n=52)**
Males				
Week 14	0	0	0	0
Week 21	0	1	0	0
Week 38	1	2	1	2
Week 52	3	7	3	4
Week 65	9	17	13	8
Total mortality	20	25	23	19
ppm	**0 (n=52)**	**120 (n=52)**	**700 (n=52)**	**[a]4000 (n=52)**
Females				
Week 14	0	0	0	1
Week 21	0	0	0	*4
Week 38	1	1	0	4
Week 52	7	3	2	7
Week 65	11	7	7	12
Total mortality	15	12	15	20

Data obtained from pages 66-71 of the study report.
* Significantly different from control, $p \leq 0.05$
[a] The dose was changed from 4000 ppm to 3000 ppm at week 24 and from 3000 ppm to 2000 ppm at week 44.
Statistics provided within the study report and not calculated by HED reviewers.

C. Discussion of Tumor Data

A slight increase in liver hemangiosarcoma was observed at 700 and 4000 ppm in males. In females, slight increases in lung adenoma and uterine cervix leiomyoma were observed at the high dose, as well as a slight decrease in uterine horn endometrial stromal polyp at the high dose (**Table 24**). Due to the small magnitude of these incidences, their relative comparison to the historical control means and ranges, and lack of a clear dose-response (in most cases), these findings were thought to reflect biological variation and **not considered treatment-related by the CARC**.

No clear increase was observed in the number of neoplasms or the number of animals with neoplasms in the treated groups when compared to control.

Historical control data were provided in the study report, and were from the conducting laboratory. There were 13 studies which spanned from 2001 to 2009. The historical control data presented in the table below were recalculated to only include studies that were within 5 years of this study, narrowing it down to 8 studies (**Table 24**).

Table 24. Afidopyropen – Mouse Carcinogenicity Study (MRID No. 49688985)

Summary of neoplastic lesions

ppm	Hist. Cont.[c]	0	120	700	4000
Males					
Liver: hemangiosarcoma (malignant neoplasm)	3.3% (0-8.9%)	1/32 (3%) (terminal kill) 1/52 (2%) (all animals)	0/27 (terminal kill) 1/52 (2%) (all animals)	2/29 (7%) (terminal kill) 3/52 (6%) (all animals)	0/33 (terminal kill) 3/52 (6%) (all animals)
No. of benign & malignant neoplasms		44	39	45	53
No. of animals with benign & malignant neoplasm(s)		29/52 (56%)	29/52 (56%)	30/52 (58%)	35/52 (67%)
ppm	**Hist. Cont.[c]**	**0**	**120**	**700**	**[a]4000**
Females					
Lung: adenoma (benign)	10.4% (3.8-17.9%)	3/37 (8%) (terminal kill) 3/52 (6%) (all animals)	2/8[b] (25%) (terminal kill) 2/20[b] (10%) (all animals)	1/4[b] (25%) (terminal kill) 1/19[b] (5%) (all animals)	6/32 (19%) (terminal kill) 6/52 (11%) (all animals)
Uterine horn: endometrial stromal polyp (benign)	4.0% (0-11.5%)	5/37 (14%) (terminal kill) 5/52 (10%) (all animals)	0/8[b] (terminal kill) 0/20[b] (all animals)	1/11[b] (9%) (terminal kill) 1/26[b] (4%) (all animals)	*0/32 (terminal kill) 1/52 (2%) (all animals)
Uterine cervix: leiomyoma (benign – all animals)	0.47% (0-3.8%)	0/52	0/12[b]	0/16[b]	2/52 (4%)
No. of benign & malignant neoplasms		43	27	30	32
No. of animals with benign & malignant neoplasm(s)		28/52 (54%)	26/52 (50%)	23/52 (44%)	23/52 (44%)

Data obtained from pages 110-121 of the study report

[a] The dose was changed from 4000 ppm to 3000 ppm at week 24 and from 3000 ppm to 2000 ppm at week 44.

[b] Examined in the animals that showed macroscopic lesions. Not subjected to statistical analysis.

[c] Historical control data were obtained from pages 606-612 of the study report and recalculated for this table. Means are presented as well as ranges.
* Significantly different from control, p≤0.05
** Significantly different from control, p≤0.01

D. Non-Neoplastic Lesions

In males, the incidence of centrilobular hepatocellular fatty change was decreased at 700 ($p \leq 0.05$) and 4000 ppm ($p \leq 0.01$), the incidence of centrilobular hepatocellular hypertrophy was increased at 4000 ppm ($p \leq 0.01$), and the incidence of secreted material depletion in granular ducts in the submandibular gland was increased at 4000 ppm ($p \leq 0.05$).

In females, a number of lesions were observed at 4000 ppm, notably a decrease in hematopoiesis in the bone marrow (sternum and femur; $p \leq 0.05$), increases in atrophy of the spleen ($p \leq 0.01$), and fibrosis of the cardiac muscles in the heart ($p \leq 0.01$). Vacuolation in the heart ($p \leq 0.01$), parietal cells in the glandular stomach ($p \leq 0.05$), hepatocytes in the liver ($p \leq 0.01$), and tubular cells in the kidney ($p \leq 0.01$) were also observed (not all data shown in Table 25).

Additionally, in females, there were increases in a number of parameters at 4000 ppm that were considered to be treatment-related but that did not have statistical significance. Notably, there were increases in the incidence of apoptosis in lymphocytes in the thymus and lymphoid follicle in the lymph nodes (cervical and mesenteric), vacuolation of neutrophil in the cortex and the choroid plexus epithelium in the cerebrum, and vacuolation of glial cell in the gray matter in the spinal cord (cervical, thoracic and lumbar).

It is worth noting that secreted material depletion in granular ducts in males may be an indication of decreases in testosterone levels[1]. The toxicological significance of this lesion was uncertain given that there were no findings related to changes in testosterone levels (**Table 25**).

[1] Botts, S., Jokinen, M., Gaillard, E.T., Elwell, M.R., and Mann, P.C.: Salivary, Harderian, and Lacrimal Glands. In: Pathology of the Mouse, edited by Maronpot, R.R., pp.49-79, Cache River Press, Vienna, IL, 1999.

Table 25. Afidopyropen – Mouse Carcinogenicity Study (MRID No. 49688985)

Relevant Non-Neoplastic Lesions

ppm	0	120	700	4000
Males				
Liver: fatty change, hepatocyte, centrilobular	13/32 (terminal kill) 14/52 (all animals)	6/27 (terminal kill) 10/52 (all animals)	*4/29 (terminal kill) **4/52 (all animals)	**1/33 (terminal kill) **1/52 (all animals)
Liver: hypertrophy, hepatocyte, centrilobular	0/32 (terminal kill) 0/52 (all animals)	0/27 (terminal kill) 0/52 (all animals)	0/29 (terminal kill) 0/52 (all animals)	**17/33 (52%) (terminal kill) **18/52 (35%) (all animals)
Sub mandibular gland: decreased secreted material in granular ducts	5/35 (14%) (terminal kill) 13/52 (25%) (all animals)	6/27 (22%) (terminal kill) 16/52 (31%) (all animals)	5/29 (17%) (terminal kill) 17/52 (33%) (all animals)	*13/33 (39%) (terminal kill) 20/52 (38%) (all animals)
ppm	**0**	**120**	**700**	**[a]4000**
Females				
Bone marrow (sternum): hematopoiesis, decreased	0/37 (terminal kill) 0/52 (all animals)	0/0 (terminal kill) 0/12[b] (all animals)	0/0 (terminal kill) 0/15[b] (all animals)	0/32 (terminal kill) *5/52 (all animals)
Bone marrow (femur): hematopoiesis, decreased	0/37 (terminal kill) 0/52 (all animals)	0/0 (terminal kill) 0/12[b] (all animals)	0/0 (terminal kill) 0/15[b] (all animals)	0/32 (terminal kill) *5/52 (all animals)
Spleen: atrophy	0/37 (terminal kill) 0/52 (all animals)	0/6 (terminal kill) 0/18[b] (all animals	0/3 (terminal kill) 0/18[b] (all animals)	0/32 (terminal kill) **10/52 (all animals)
Thymus: increased apoptosis, lymphocyte, cortex	0/37 (terminal kill) 0/52 (all animals)	0/12[b] (terminal kill) 0/23[b] (all animals)	0/7[b] (terminal kill) 0/22[b] (all animals)	0/32 (terminal kill) 4/52 (all animals)
Lymph node (cervical): increased apoptosis, lymphoid follicle	0/37 (terminal kill) 0/52 (all animals)	0/2[b] (terminal kill) 0/14[b] (all animals)	0/2[b] (terminal kill) 0/17[b] (all animals)	0/32 (terminal kill) 3/52 (all animals)
Lymph node (mesenteric): increased apoptosis, lymphoid follicle	0/37 (terminal kill) 0/52 (all animals)	0/2[b] (terminal kill) 0/14[b] (all animals)	0/1[b] (terminal kill) 0/16[b] (all animals)	0/32 (terminal kill) 4/52 (all animals)

Data obtained from pages 122-155 of the study report.

[a] The dose was changed from 4000 ppm to 3000 ppm at week 24 and from 3000 ppm to 2000 ppm at week 44.

[b] Examined in the animals that showed macroscopic lesions. Not subjected to statistical analysis

* Significantly different from control, $p<0.05$; ** Significantly different from control, $p\leq0.01$

E. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate and not excessive based on body weight loss and histopathology. There were no significant findings for absolute body weight in males, but there was a decrease in absolute body weight in females (≥14%) at 4000 ppm throughout most of the study period. Food consumption was statistically significant decreased at week 1 in both sexes at 4000 ppm. Food efficiency was also found to be decreased in females at the high dose from week's 9-12.

In females at 4000 ppm, clinical examination identified increases in prone body position and bradypnea, and a decrease in spontaneous motor activity. There were increases in hematological blood parameters, absolute and relative spleen and ovary weights, an increase in pale colored liver in high dose females. Histopathology results for male and females dosed at 4000 ppm were discussed above and add support to the adequacy of dosing in the mouse carcinogenicity study.

IV. TOXICOLOGY

1. Metabolism

Afidopyropen (^{14}C-BAS 440) is rapidly absorbed and distributed to tissues following a single gavage administration at low and high dose levels to rats. After administration of 3 mg/kg bw, plasma C_{max} reached 0.097-0.171 µg-equiv/mL with T_{max} occurring at 0.5 hours. Following administration of 300 mg/kg bw, plasma C_{max} reached 22-24 µg-equiv/mL with T_{max} occurring at 2-4 hours. Afidopyropen was distributed widely to tissues, with highest levels of radioactivity observed in the GI tract and contents, liver, adrenals and kidney in both sexes at 0.5 hours and 2 hours for low and high dose administration, respectively. In females of the high dose group, the pituitary also had a high level of radioactivity and notable concentrations were also observed in the uterus and ovaries. The elimination half-life in whole blood was 1 – 2.5 hours following administration of 3 mg/kg/bw and 15 – 16 hours following administration of 300 mg/kg/bw. Half-lives were similar between sexes at 3 mg/kg/bw and slightly increased in males at the high dose. Radioactivity levels were notably reduced after 96 hours with no significant differences noted between sexes.

In a separate biliary excretion experiment, bile-duct cannulated Wistar and Fisher rats were dosed *via* gavage with afidopyropen (^{14}C-BAS 440) at dose levels of 3 or 300 mg/kg/bw. At 3 mg/kg/bw, absorption from urine and bile was calculated to be 57% for both sexes in Wistar rats and 67-70% in Fisher rats. At 300 mg/kg/bw, absorption for Wistar rats was 57-60% and for Fisher rats 71-72% for both sexes. Bioavailability between males and females was not significantly different. It can be concluded that the feces are the major route of elimination for afidopyropen.

In an additional study, plasma kinetics were examined after 14 days of dietary administration of non-radiolabeled afidopyropen followed by a single oral gavage of radiolabeled afidopyropen (^{14}C-BAS 440) to female rats. Target concentrations for both the multiday non-radiolabeled diet and single radiolabeled gavage doses were 3, 15, or 50 mg/kg/bw. Afidopyropen was rapidly absorbed from the GI tract and maximum plasma concentrations were reached by 1-hour post-dose for the low and mid-dose groups and 2 hours for the high-dose group. Detection in the feces was 85%, 90% or 65% of the administered dose for the 3, 15, or 50 mg/kg/bw groups, respectively. With increasing dose, the amount of radioactivity detected in the urine increased (0.917% at 3 mg/kg/bw, 1.3% at 15 mg/kg/bw, and 1.64% at 50 mg/kg/bw). The majority of the excretion occurred between 8-48 hours, irrespective of dose.

Afidopyropen was extensively metabolized and there were no gender-related differences in metabolism. The main biotransformation reactions were: hydrolytic loss of one or both CPCA ester moieties, N-oxidation at the pyridine ring, hydroxylation of one of the methyl groups, and conjugation of hydroxyl groups of the metabolites.

2. Mutagenicity

There is no concern for mutagenic activity with afidopyropen. A battery of mutagenicity studies

including, bacterial reverse mutation, *in vitro* mammalian cell gene mutation, *in vitro* mammalian chromosome aberration, and mouse erythrocyte micronucleus all produced negative results (**Table 26**).

Table 26: Summary of Genotoxicity Studies for Afidopyropen

Bacterial Reverse Mutation Test	870.5100 Afidopyropen (95.74%) MRID 49688972 (2009) Acceptable/Guideline Doses up to 5000 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
	870.5100 Afidopyropen (94.5%) MRID 49688973 (2015) Acceptable/Guideline Doses up to 5300 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
	870.5100 Afidopyropen (97.3%) MRID 49688974 (2015) Acceptable/Guideline Doses up to 5200 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
	870.5100 Afidopyropen (90.0%) MRID 49688975 (2015) Acceptable/Guideline Doses up to 5600 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
	870.5100 Afidopyropen formulation (9.6% a.i.) MRID 49689205 (2014) Acceptable/Guideline Doses up to 5000 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
In vitro mammalian cell gene mutation test	870.5300 Afidopyropen (95.54%) MRID 49688977 (2015) Acceptable/Guideline Trial 1: 4h at concentrations of 0, 4.7, 9.4, 18.8, 37.5, 75, 150, 300 µg/mL (−S9) and 0, 2.3, 4.7, 9.4, 18.8, 37.5, 75, 150, or 300 µg/mL (+S9) Trial 2: concentrations of 0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, or 250 µg/mL (±S9)	Negative**.** There was no evidence of gene mutations with or without activation.
In vitro mammalian	870.5375 Afidopyropen (95.74%)	

chromosome aberration test	MRID 49688976 (2009) Acceptable/Guideline 0, 78.1, 156, 313, 625 µg/mL for 6 h with an 18-h recovery period (±S9) 0, 30.9, 46.3, 69.4, 104,156 µg/mL for 24 h with no recovery period (–S9)	Negative.
Mammalian Erythrocyte Micronucleus-Mouse	870.5395 Afidopyropen (90.0%) MRID 49688978 (2016) Acceptable/Guideline ♂: 0, 350, 700, 1400 mg/kg bw	Negative. There was no significant increase in the frequency of micro-nucleated polychromatic erythrocytes.
	870.5395 Afidopyropen (95.7%) MRID 49688979 (2015) Acceptable/Guideline ♂: 0, 250, 500,1000 mg/kg bw	Negative. There was no significant increase in the frequency of micro-nucleated polychromatic erythrocytes.
	870.5395 Afidopyropen (95.74%) MRID 49688980 (2009) Acceptable/Guideline ♂: 0, 500, 1000, 2000 mg/kg bw	Negative. There was no significant increase in the frequency of micro-nucleated polychromatic erythrocytes.
	870.5395 Afidopyropen (formulation containing 9.6% a.i.) MRID 49689220 (2015) Acceptable/Guideline ♂: 0, 500, 1000, 2000 mg/kg bw	Negative. There was no significant increase in the frequency of micro-nucleated polychromatic erythrocytes.
Bacterial Reverse Mutation test - ME5343-T7 (plant metabolite)	870.5100 Afidopyropen metabolite (ME5343-T7; 97.3%) MRID 49688999 (2014) Acceptable/Guideline Doses up to 5200 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
	870.5100 Afidopyropen metabolite (ME5343-T7; 98.52%) MRID 49688998 (2012) Acceptable/Guideline Doses up to 5000 µg/plate (with and without activation)	Negative. There was no evidence of induced reverse mutations with or without activation.
In vitro mammalian cell	870.5300 Afidopyropen metabolite (ME5343-T7; 97.3%) MRID 49689004 (2015) Acceptable/Guideline	Negative. There was no evidence of gene

gene mutation test - ME5343-T7 (plant metabolite)	Trial 1: 0, 9.38, 18.75, 37.5, 75, 150, 300 µg/mL (±S9, 4-h exposure); Trial 2: 0, 9.38, 18.75, 37.5, 75, 150, 300 µg/mL in (-S9, 24-h exposure); and 0, 12.5, 25, 50, 100, 200, or 300 µg/mL (+S9, 4-h exposure)	mutations with or without activation.
Mammalian Erythrocyte Micronucleus-Mouse - ME5343-T7 (plant metabolite)	870.5395 Afidopyropen metabolite (ME5343-T7; 97.3%) MRID 49689003 (2015) Acceptable/Guideline ♂: 0, 500, 1000, 2000 mg/kg bw	The PCE:NCE ratios were decreased compared to the control at all doses at both time points, indicating that the test material was slightly toxic to the bone marrow. No significant increases in the MPCE frequency were observed at any dose.
Micronucleus Assay in Human Lymphocytes *in Vitro* - ME5343-T7 (plant metabolite)	OCSPP 870 Supplemental; OECD 487 Afidopyropen metabolite (ME5343-T7; 97.3%) MRID 49689135 (2015) Acceptable/Non-Guideline Trial 1: 4 h at nominal concentrations of 0, 4.0, 8.0, 16.1, 32.1, 64.2, 128.4, 256.9, 513.8, 1027.5, 2055 µg/mL (±S9) Trial 2: 20 h at nominal concentrations of 0, 4.0, 8.0, 16.1, 32.1, 64.2, 128.4, 256.9, 513.8, 1027.5, 2055 µg/mL (−S9) and 0, 8.0, 16.1, 32.1, 64.2, 128.4, 256.9 µg/mL (+S9)	Negative. No biologically-relevant increases in the percentage of micronucleated cells were observed at any concentration in the presence or absence of S9 activation in either trial.

3. Structure-Activity Relationship

There are no structurally similar chemicals for afidopyropen. According to HED's Integrated Structure, Toxicology, Endpoints and Properties (ISTEP) database, afidopyropen does not contain information on similar chemical classes. According to the Alan Wood database, afidopyropen is an "unclassified insecticide". ChemIDplus did not provide any structurally related pesticides.

4. Subchronic and Chronic Toxicity

Subchronic and chronic toxicity studies conducted on the rat and mouse are summarized below. It should be noted that the submitted afidopyropen toxicology studies were reviewed jointly by the EPA, USA and the Pest Management Regulatory Agency (PMRA), Canada. Under the summary of each study, the "EPA Reviewer Comments" are where EPA and PMRA conclusions differed.

a) Subchronic Toxicity

90-day Oral Rat Subchronic Toxicity Study (MRID 49688967)

In a subchronic toxicity study, ME5343 (95.74% a.i.) was administered to 10 Fischer (F344/DuCrlCrlj) rats/sex/dose via the diet at dose levels of 0, 150, 300, 1000 or 3000 ppm (8.9/10.2, 18.3/20.4, 61.0/68.2, 182.0/197.0 mg/kg bw/day ♂/♀) for 90-days.

At 300 ppm, a decrease in triglyceride was observed in males.

At 1000 ppm and higher, an increase in relative liver weight was observed in both sexes. In males, increases in urinary urobilinogen and relative kidney weight were observed. In females, decreases in food consumption and absolute heart weight were observed as well as increases in blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, potassium, absolute liver weight, relative spleen weight, and absolute and relative thymus weight. Additionally, in females, vacuolar changes in hepatocytes and myocardium were observed.

At 3000 ppm in both sexes, there were decreases in hematocrit, red blood cells, hemoglobin, and total bilirubin, as well increases in albumin/globulin ratio, alkaline phosphatase, and absolute spleen weight. Additionally, cloudiness of the liver and congestion of the spleen was observed in both sexes. In males, there were decreases in grip strength of the hind limbs, food consumption, and urinary casts, as well as increases in in platelets, blood urea nitrogen, relative spleen weight, and absolute liver weight. Additionally, in males, vacuolar changes in hepatocytes and myocardium were observed. In females, decreases in body weight, triglyceride, relative heart weight, and absolute and relative ovary and uterus weights were observed, as well as an increase in glucose.

EPA Reviewer Comments – The small magnitude of change in hematology parameters at 1000 ppm were not considered adverse in either sex. The increase in liver and kidney weight in males was not considered adverse without corresponding histopathology.

90-day Oral Mouse Subchronic Toxicity Study (MRID 49688968)

In a subchronic toxicity study, ME 5343 Technical (95.74% a.i.) was administered to 10 (SPF) ICR [Crlj:CD1(ICR)] mice /sex/dose in the diet at dose levels of 0, 150, 500, 2000, or 6000 ppm (0/0, 20.9/25.2, 68.9/82.8, 285/327, or 819/919 mg/kg bw/day, in males and females, respectively) for 90 days.

In the 6000 ppm dose group, five of ten females died during the course of the study (one was found dead in week 2, four were killed *in extremis* at weeks 4, 10, or 13). A statistically significant increase in cumulative death rate was noted at weeks 10-13 in females when compared to the control group. The females killed *in extremis* showed significant increases in incidences of lateral position, decreased spontaneous motor activity, and bradypnea when compared to the control group. Some of these females also showed soiled or wetted fur in the genital region and/or piloerection. There were no unscheduled deaths or clinical signs of toxicity observed in high dose males, or in the groups receiving ≤ 2000 ppm of the test material. No treatment-related effects were noted on the body weight or body weight gain of any treated animals as compared to controls. Food consumption was decreased in high dose males and females in week 1 of the study.

Blood analyses revealed increased red cell distribution width, and decreased platelet and lymphocyte counts in males and females in the 6000 ppm dose groups. In high dose males,

hemoglobin distribution width and neutrophil counts were increased, and in high dose female's hemoglobin levels were decreased, and monocyte and eosinophil counts were increased. With respect to clinical chemistry parameters, total, direct and indirect bilirubin levels were statistically significantly increased in male and female animal dosed at 2000 ppm and above. AST and ALT levels were statistically significantly increased in high dose males and triglyceride levels were relatively high in females receiving ≥ 2000 ppm.

In high dose females, both absolute and relative liver weight was increased and ovary weight was decreased. Absolute and relative spleen weight was increased in high dose males and females treated with 2000 and 6000 ppm.

During gross necropsy, soiled fur in the external genital region of three high dose females was observed along with accentuated lobular pattern and pale color in the livers of one to two high dose females. Histopathological examinations of the livers of males in the high dose group revealed a statistically significant increase in single cell necrosis of hepatocytes, and in high dose males and females (surviving to study termination) a statistically significant increase in incidences of centrilobular hepatocellular hypertrophy. In high dose females killed in extremis or found dead, there were also observations of diffuse hepatocellular vacuolation and focal hepatocellular necrosis. One surviving female also exhibited single cell necrosis of hepatocytes. Males in the 6000 ppm group had a significant increase in incidence of secreted material depletion in granular ducts of the submandibular gland. Females found dead or killed *in extremis* in the 6000 ppm group showed vacuolation of multiple tissues (cardiac muscle fiber, parietal cell of the glandular stomach, hepatocytes, proximal tubular cell of the kidney, mucosal epithelial cell of the urinary bladder, neuropil in the cerebral cortex, choroid plexus epithelium and glial cell in gray matter of spinal cord). Decreases of hematopoiesis in the bone marrow (sternum and femur) and increases of apoptosis in lymphoid tissues (spleen, thymus and cervical/mesenteric lymph nodes) were observed in the females found dead or killed *in extremis* in the 6000 ppm group.

90-day Subchronic Rat Neurotoxicity Study (MRID 49688997)

In a subchronic neurotoxicity study, groups of 10 Wistar Crl:WI(Han) rats/sex/dose were administered afidopyropen (94.54% a.i.; Batch # 080722) in the diet at dose levels of 0, 300, 1000, or 4000 ppm (equivalent to 0/0, 20/24, 73/92, and 396/438 mg/kg bw/day in males/females) for 13 weeks. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was conducted on 10 rats/sex/group pre-dose and on Days 1, 22, 50, and 85. At study termination (Day 91), all rats were anesthetized and perfused *in situ,* and brain weights were recorded. The tissues from the perfused animals in the control group and high-dose group (4000 ppm) were subjected to histopathological evaluation of central and peripheral nervous system tissues. Acceptable positive control data were provided.

No compound-related effects were observed for mortality, clinical signs of toxicity, FOB observations, motor activity assessments, brain weights, or gross pathology and neuropathology in either sex.

Body weight in the 4000 ppm males was decreased ($p<0.05$/$p<0.01$) for nearly all study days

measured (study days 7-70 and study day 91). Most values reach ≥10% reduction in body weight. The body weights determined prior to FOB assessments also were decreased in the males by 10-14%. Mean body weight gains (BWG) in the males were decreased throughout the study [↓18-43%; ↓20% for overall (Days 0-91) BWG].

This subchronic neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OCSPP 870.6200b; OECD 424) for a subchronic neurotoxicity study in rats.

b) Chronic Toxicity

1-year Rat Oral Toxicity Study; Low Dose (MRID 49688981)

In a chronic toxicity study, ME5343 Technical (95.74% a.i.) was administered to 24 Fischer 344 rats/sex/dose in the diet at dose levels of 0, 75, 150, 300, or 1000 ppm (0, 3.7, 7.3, 15, or 48 mg/kg bw/day in males and 0, 4.4, 8.9, 18, or 56 mg/kg bw/day in females, respectively) for one year. Note that a second 1-year study in rats (MRID 49688982) was conducted at higher doses using dietary dose levels of 0, 1000, and 3000 ppm as a supplement to this study.

No treatment-related clinical signs of toxicity or effects on body weight, food consumption (according to the EPA reviewer), ophthalmoscopic or urinalysis parameters were observed. One male in the 150 ppm group died at test week 30 due to a spontaneously occurring nephroblastoma.

At the high dose of 1000 ppm, increased platelet counts and decreased triglyceride levels were observed in both sexes. A slight reduction in alanine aminotransferase was also noted but in males only. Females from the 1000 ppm dose group exhibited slightly reduced food consumption (although not considered adverse by the EPA reviewer) and increased alkaline phosphatase levels. Upon histological examination of tissues, high-dose females demonstrated an increased incidence of slight vacuolar change of hepatocytes and myocardial cells. The vacuolar change was considered likely to be the result of lipid deposition. Slight vacuolar change of hepatocytes was also observed at the 300 ppm dose level, but in two females (8%) only. Given the low incidence of this finding, and the absence of other treatment-related effects at this dose level, the vacuolar change in the liver of the 300 ppm females is considered to be non-adverse. Given the uterine findings throughout the afidopyropen database it should also be noted that high dose females had a decrease in absolute (↓10%) and relative (↓9%) uterine weight.

1-year Rat Oral Toxicity Study; High Dose (MRID 49688982)

In a chronic toxicity study, BAS 440 I (95.74% a.i.) was administered to 24 Fischer 344 rats/sex/dose in the diet at dose levels of 0, 1000, or 3000 ppm (0, 48, or 143 mg/kg bw/day in males and 0, 57, or 161 mg/kg bw/day in females, respectively) for one year. This study was conducted subsequent to a previous 1-year toxicity study in rats (PMRA 2627860; MRID 49688981) that included dose levels of 0, 75, 150, 300, and 1000 ppm.

No treatment-related clinical signs of toxicity or effects on body weight or ophthalmoscopic parameters were observed. Food consumption was slightly reduced in females from the 1000 ppm dose level and in both sexes at 3000 ppm; females from the 3000 ppm dose group also exhibited an increase in food conversion efficiency. One male in the 1000 ppm group died at test week 27 without any obvious lesions related to the cause of death. One female is the 1000 ppm group was euthanized in a moribund state at week 41. This state was attributed to an adenocarcinoma of the mammary gland.

At doses of 1000 and 3000 ppm, both sexes exhibited increased platelet counts, decreased triglyceride levels, and increased absolute and relative liver, spleen, and kidney weights. Increased alkaline phosphatase levels were noted in males at 1000 and 3000 ppm and in females at 3000 ppm only. Other changes noted in clinical chemistry parameters included decreases in bilirubin levels in females from the 1000 and 3000 ppm dose groups, decreases in aspartate aminotransferase and alanine aminotransferase in 3000 ppm males, and increased glucose levels in 3000 ppm females. At 3000 ppm there was an increase in potassium levels in females.

Decreases in red blood cell parameters (red blood cell counts, hemoglobin content, and hematocrit) were slight in nature and observed only in males from the 3000 ppm dose group. Increased reticulocyte counts were also observed in males at this dose level.

At 3000 ppm only, alterations in urinalysis parameters were noted and included increased levels of bilirubin in the urine of males and females, and in females only a decrease in urine volume, an increase in urine specific gravity, and increased amounts of protein and ketones in urine.

No treatment-related gross or histological lesions were noted in males. Pathological findings in females at the 1000 and 3000 ppm dose levels included vacuolar change of the myocardium, accompanied by decreased absolute and relative heart weight, as well as vacuolar change in the liver and decreased zymogen granules in the acinar cells of the pancreas. The vacuolar change observed in the heart and liver increased in severity from slight at 1000 ppm to moderate at 3000 ppm, and was considered to be due to lipid deposition. Additional pathological findings noted only in females from the 3000 ppm dose group included discoloration of the liver, slight hyperplasia of the bile duct, slight foci or altered cells of the liver (determined to be basophilic and of the tigroid type), and slight focal hyperplasia of the anterior lobe of the pituitary. Males in both the 1000 and 3000 ppm groups also exhibited increased relative and absolute adrenal, thyroid, testes, and epididymal weight. Females exhibited a decrease in absolute and relative uterine weight and an increase in pituitary weight at 1000 and 3000 ppm. Females at 3000 ppm exhibited increased relative and absolute thyroid weight.

EPA Reviewer Comments – At 1000 ppm the increase in kidney weight was not considered adverse given the small magnitude and change and no corresponding histopathology. The change in spleen weight is also not considered adverse in the absence of corresponding histopathology. Given the uterine tumors noted in other rat carcinogenicity studies, the decreased uterine weight at 1000 ppm was considered adverse.

Chronic Carcinogenicity Study in the Rat; Low Dose (MRID 49688983)

In a carcinogenicity study, ME5343 technical (95.74% a.i.) was administered to 50 Fischer rats/sex/dose via the diet at dose levels of 0, 100, 300, or 1000 ppm (0/0, 4.4/5.3, 12.9/15.5, or 42.7/50.8 mg/kg bw/day ♂/♀) for 2 years.

There were no adverse effects on clinical signs, mortality, body weight, or hematological parameters in either sex at any of the tested dose levels.

In males, increases in absolute and relative kidney, liver and adrenal gland weights were observed at 1000 ppm.

In females, there was a slight decrease in food consumption and an increase in absolute and relative uterine weight at 1000 ppm. Additionally, at 1000 ppm, there were increases in the incidence of hyperplasia of the bile duct in the liver and uterine adenocarcinoma.

EPA Reviewer Comments – In the absence of corresponding histopathology, the increase in kidney and liver weight in males is not considered adverse. Uterine weight is still considered adverse given the slight endometrial hyperplasia that is also present within the study and also seen in the second rat carcinogenicity study (MRID 49688984).

Chronic Carcinogenicity Study in the Rat; High Dose (MRID 49688984)

In a carcinogenicity study, BAS 440 I (95.74% a.i.) was administered to 50 Fischer rats/sex/dose via the diet at dose levels of 0, 1000, or 3000 ppm (0/0, 41.6/50.4, or 128.2/146.9 mg/kg bw/day in ♂/♀) for 2 years.

An increase in mortality was noted at 3000 ppm in females. At 1000 ppm and higher, a decrease in food consumption was observed in both sexes. The reductions at 1000 ppm in both sexes were minimal and not considered adverse. There were no changes in hematological parameters. In males, an increase in the absolute and relative epididymides weight was observed at 1000 and 3000 ppm. In females at 1000 ppm, there was a slight increase in relative liver weight, and decreases in absolute heart weight and absolute and relative ovary weight. Additionally, in females at ≥1000 ppm, there were increases in the incidences of opacity of unilateral lens, adenocarcinoma in the uterus, and bile duct hyperplasia in the liver.

At 3000 ppm in both sexes, there was a decrease in body weight and body weight gain, increases in absolute and relative liver, spleen and kidney weight, and in the incidence of pituitary cysts. In high dose males there was a decrease in absolute and relative adrenal weight and in females a decrease in relative adrenal weight. In males, there were increases in the incidences of mandibular lymph node cysts, sinus dilatation in the mandibular lymph node, foci of altered cells in the liver, and the number of sperm in the epididymis. In females, there was an increase in absolute and relative uterus weight. Additionally, in females there were increases in the incidences of chronic progressive nephrosis in the kidney, and endometrium hyperplasia in the uterus, as well as decreases in duct dilation of the mammary gland, and zymogen granules in the pancreas.

EPA Reviewer Comments – In the absence of corresponding histopathology the decrease in absolute and relative epididymides weight is not considered adverse. The decrease in absolute body weight in males never reached adversity at 3000 ppm. The increase in pituitary cysts at 1000 ppm in females was considered adverse.

Chronic Carcinogenicity Study in the Mouse (MRID 49688985)

In a carcinogenicity study, ME5343 Technical (95.74 % a.i.) was administered to 52 ICR mice/sex/dose via the diet at dose levels of 0, 120, 700 and 4000 ppm (0/0, 13.3/12.9, 78.7/75.8, 445/333 mg/kg bw/day ♂/♀) for 78 weeks. In the high dose group in females, the dose was changed to 3000 ppm at week 24, and then to 2000 ppm at week 44 due to death or moribundity.

In females at 4000 ppm, there was an increase in mortality observed after 14 weeks of treatment and the dose was reduced to 3000 ppm at week 24 in females. Two additional females were killed in extremis at week 41 and the dose was further reduced to 2000 ppm at week 44. The mortality rate became comparable to that of the control, and no significant difference was noted in the final mortality at the termination of the treatment groups. Clinical examination identified increases in prone body position and bradypnea, and a decrease in spontaneous motor activity in females at 4000 ppm. There were no significant findings for absolute body weight in males, but there was a decrease in absolute body weight in females (≥14%) throughout most of the study period. Food consumption was statistically significant decreased at week 1 in both sexes at 4000 ppm. Food efficiency was also found to be decreased in females at the high dose from week's 9-12.

There were increases in white blood cell count, lymphocyte count, large unstained cell count, absolute and relative spleen and ovary weights, an increase in pale colored liver in high dose females. Histopathology examination identified decreased hematopoiesis in bone marrow (sternum and femur), atrophy of the spleen, fibrosis of cardiac muscle in the heart, apoptosis in lymphocytes in the thymus and lymphoid follicle in the lymph nodes (cervical and mesenteric) in the high dose males. The high dose males also exhibited a decrease in fatty change in hepatocytes, and increase in centrilobular hepatocyte hypertrophy and decreased secreted material in the granular ducts of the sub mandibular gland. Additionally, there was vacuolation of i) cardiac muscle in the heart, ii) parietal cell in the glandular stomach, iii) hepatocytes in the liver, iv) proximal tubule cell in the kidney, v) mucosal epithelial cell of the urinary bladder, vi) neutrophil in the cortex and the choroid plexus epithelium in the cerebrum, and vii) glial cell in the gray matter in the spinal cord (cervical, thoracic and lumbar) also found in the high dose males.

There were no treatment-related neoplasms identified in this study.

EPA Reviewer Comments – Only decreases in absolute body weight in females was considered adverse at 4000 ppm. Males did not reach adverse effect levels.

5. Proposed Mode of Action for Rat Uterine Tumors

The Registrant submitted 12 studies to support a MOA for uterine tumors in female rats in addition to an overview document detailing a human relevance framework analysis (MRID 49689130).

a. Postulated Mode of Action for Uterine Tumors in Female Rats

The following dopamine enhancement mode of action has been postulated by the Registrant (MRID 49689130) for afidopyropen-induced rat uterine tumors through the following Key Events:

1) **Key Event #1**: Agonism of dopamine receptor
2) **Key Event #2**: Decreased serum prolactin (Prl) levels
3) **Key Event #3**: Decreased corpus luteum support leading to decreased production of progesterone and resulting estrogen dominance
4) **Key Event #4**: Altered reproductive senescence in aged rats
5) **Key Event #5**: Endometrial hyperproliferation
6) **Key Event #6**: Promotion of uterine adenocarcinomas

Data supporting Key Event #1 (Agonism of dopamine receptor):

1. *In silico* prediction (MRID 49689007)

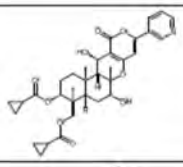
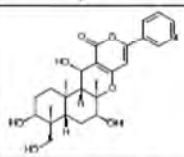
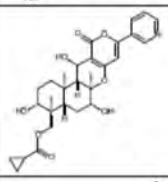
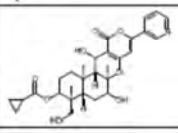
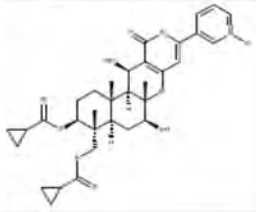
The molecular structures of afidopyropen and its metabolites M4401001, M4401002, M4401003 and M4401017 were submitted to *in-silico* activity prediction models of the human dopamine D2 receptor (hd2-) and dopamine transporter (hdat-). These in-house (BASF) QSAR models predict the strength of inhibition of the above mentioned targets based on the structural similarity of the input molecules to those with a reported measured inhibition value (refer to Anger *et al*., 2014 for methodical details). The models refer to the *in vitro* activity at the target only, not taking into account pharmacokinetic effects like distribution or metabolizing of the compounds *in vitro.*

The prediction output can be found in **Table 27**. It contains a quantitative activity prediction in form of a pIC50 value (=-log(IC50) in molar concentration) along with an error approximation for each input compound. The activity classification (i.e. borderline, inactive) is chosen as a comparison to the underlying activity distribution of measured compounds used in training of the models (i.e. an inactive classification means that the compound is predicted to be less active than the average of compounds measured). An out-of-domain compound means a reliable prediction cannot be made because the compounds in the training set are not similar enough.

Overall, the predictions for afidopyropen and its metabolites are similar. The only exception is metabolite M4401017 whose prediction for the dopamine D2 receptor (hd2-) is out-of-domain as it is structurally more distant from the rest of the compounds. For the other metabolites, and afidopyropen, high inhibition values are predicted for the dopamine receptor in the range of 10^{-8} molar. The predictions are labeled as borderline because the high uncertainty of the predictions does not allow a clear classification above the cut-off (median of the distribution). However, the

in silica predictions suggest a possible modulation of this target. An inhibition of the dopamine transporter (hdat-) by the investigated compounds seems unlikely given the rather low pIC50 predictions for this target.

Table 27: In-silico activity predictions for afidopyropen and its main metabolites at the dopamine receptor (hd2-) and the dopamine transporter (hdat-).

Compound ID	Structure	Activity	
		Dopamine Receptor (hd2-)	Dopamine Transporter (hdat-)
Afidopyropen (BAS 440 I)		7.23±0.99 **Borderline Activity**	5.55±0.7 **Inactive**
M440I001		7.09±0.93 **Borderline Activity**	5.6±0.78 **Inactive**
M440I002		7.31±0.95 **Borderline Activity**	5.57±0.72 **Inactive**
M440I003		7.33±0.95 **Borderline Activity**	5.56±0.72 **Inactive**
M440I017		**Out of domain**	5.54±0.71 **Inactive**

The activity value is a quantitative prediction from QSAR models that were individually built for several relevant human off-targets. It is given as a pIC50/pEC50 value (=-log (IC50/EC50 in molar concentration)). In addition, an error estimation is given, which is calculated by an extrapolation function based on the chemical distance to the molecules in the training set of the QSAR model.

2. *In vitro* binding assays (MRID 49689011, MRID 49689012, MRID 49689013):

Afidopyropen and four rat metabolites (M440I001, M440I002, M440I003 and M440I017) were tested in a battery of screening assays measuring interaction with dopamine related receptors and functions (**Table 28**).

In radioligand binding assays neither afidopyropen nor any of the tested metabolites showed any

binding to D2s or $D2_L$ human receptors (MRID 49689012). Similarly, a D1 functional assay (measuring cyclic AMP production after stimulation of human recombinant D1) did not see a significant agonistic or antagonistic effect (i.e. in the absence or presence of dopamine stimulation) of afidopyropen or its metabolites (MRID 49689011). Taken together, these results indicate afidopyropen and its metabolites probably do not interact orthosterically (i.e. do not bind to the primary receptor site) with the D1 or D2 receptors.

Afidopyropen and the above mentioned metabolites were also tested for interaction with the human dopamine active transporter (DAT) in a radioligand binding assay (MRID 49689011). In conjunction with the assay, a dopamine uptake assay, using synaptosomes from rat striatum, served as a functional bioassay of the DAT. In both of these assays, neither afidopyropen nor its metabolites showed interaction with the DAT on a molecular or a functional level.

Two types of tissue bioassays were conducted; one measuring D1 activation and another measuring D2 activation (MRID 49689011). In the rabbit splenic artery assay (a model of D1 receptor activity), there was no significant agonist nor antagonist effect observed. In the field stimulated rabbit ear artery assay (a model of D2 receptor activity), afidopyropen and M440I002 indicated a D2 agonist-like activity (i.e. responses higher than 50%). M440I017 also showed some D2 agonist activity. The observed activity was not reversed by the addition of a dopamine antagonist (-) sulpiride. Neither afidopyropen nor its metabolites showed antagonist activity in this assay.

The field stimulated rabbit ear artery assay of the D2 receptor was repeated (MRID 49689013) with afidopyropen and M440I002 after solvent pre-treatment, again showing the D2 agonist response that was not reversed by (-) sulpiride when added at the end of the experiment. A modified protocol was then completed; after 20-minute pre-treatment with (-) sulpiride, afidopyropen and M440I002 induced a concentration-dependent decrease in the twitch contraction amplitude that was right shifted when compared to the effect after solvent pre-treatment. The Registrant concluded that this demonstrated a dopamine agonist response that was reduced by pre-treatment with a dopamine antagonist and that this effect is likely due to a high binding affinity of afidopyropen to the rabbit D2 receptor, with (-) sulpiride not being able to reverse the binding of afidopyropen. However, afidopyropen is able to partially displace the bound D2 antagonist (-) sulpiride.

Table 28: *in-vitro* dopamine receptor interaction studies

Assay	Receptor	Tested Compounds	Results	Reference
Radioligand binding	dopamine receptor ($D1_h$) agonist effect	Afidopyropen M440I001 M440I002 M440I003 M440I017	No Activity	MRID49689011
	dopamine receptor ($D1_h$) antagonist effect		No Activity	
Radioligand binding	dopamine transporter(h)		No Activity	
Dopamine uptake	synaptosomes from rat striatum		No Activity	
Tissue bioassay	Rabbit splenic artery; D1 Agonist effect		No Activity	
	Rabbit splenic artery; D1 Antagonist effect		No Activity	
D2 Tissue bioassay I	Field stimulated rabbit ear artery; D2; agonist effect		Afidopyropen and M440I002 showed D2 agonist-like effects that were not reversed by (-)Sulpiride*	
	Field stimulated rabbit ear artery; D2; antagonist effect		No Activity	
Radioligand binding	dopamine receptor ($D2_h$) agonist effect	Afidopyropen M440I002	No Activity	MRID49689012
	dopamine receptor ($D2_h$) antagonist effect		No Activity	
D2 Tissue bioassay II	Field stimulated rabbit ear artery; D2; agonist effect; Standard protocol		Both molecules showed D2 agonist-like effects that were not reversed by (-)Sulpiride*	MRID49689013
	Field stimulated rabbit ear artery; D2; agonist effect; Modified protocol; pre-incubation with (-) Sulpiride		Concentration-dependent decrease in the twitch contraction amplitude, that was reduced by pre-incubation with (-)sulpiride; Confirmed D2 agonist effect	

*(-)Sulpiride is an antagonist of the D2 receptor

CARC concluded that there is no direct evidence from the Registrant submitted studies that afidopyropen is an agonist of the dopamine receptor (Key Event #1). The results from the rabbit in vitro assays were difficult to interpret in the absence of a clear binding effect. Even though <u>multiple</u> in silico and in vitro binding assay results were presented, the overall weight-of-evidence conclusion still did not support agonism of the dopamine receptor for either parent or any metabolites tested. The strongest evidence to support Key Event #1 was from the in silico assays and it was concluded that these results were "moderate" and "possible" at best. Finally, there are no known pesticides that act via agonism of the dopamine receptor with resulting uterine tumors, making the establishment of Key Event #1 a novel event that should be supported in concrete data.

Data supporting <u>Key Event #2</u> (Decreased serum prolactin (Prl) levels):

Prolactin levels were measured in three mechanistic rat studies. In the first subchronic study (MRID 49689127), afidopyropen was administered to 10 Fischer rats in the diet at dose levels of 0, 300, 1000 or 4000 ppm (0, 21, 79, or 404 mg/kg bw/day) for 90 days. An additional control group of 10/sex were used for blood sampling and clinical parameters only. Rat prolactin levels were measured in samples taken from females in all test groups on study day 92 using an ELISA kit (Demeditec, Kiel, Germany).

Since prolactin value variation was expected to be high, the samples from both control groups were combined for comparison with the test groups. Mean serum prolactin levels were statistically significantly decreased in females from the 4000 ppm dose group in comparison to the controls (**Table 29**).

Table 29. Serum prolactin values (µg/L) in females following 92 days of treatment [a]

ppm	0[b]	300 (21 mg/kg/day)	1000 (79 mg/kg/day) [c]	4000 (404 mg/kg/day) [c]
Day 92: mean	43.27 ± 45.78	168.19 ± 243.31	152.78 ± 245.97	**10.88 ± 5.26***
Day 92: median	28.73	26.29	58.80	**10.86**

[a] Data obtained from page 125 in the study report (MRID 49689127).
[b] Values from both controls groups were combined; N=20
[c] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)
* Statistically different from diestrus, p<0.05
** Statistically different from diestrus, p<0.01

In the second subchronic study (MRID 49689128), afidopyropen was administered to 10 Fischer rats in the diet at dose levels of 0, 300, 1000 or 4000 ppm (0, 20, 60, 361 mg/kg bw/day) for 90 days. An additional control group of 10/sex were used for blood sampling and clinical parameters only. Prolactin levels were measured in samples taken from females in all test groups on study day 92 using an ELISA kit (Demeditec, Kiel, Germany).

Since prolactin value variation was expected to be high, the samples from both control groups were combined for comparison with the test groups. Mean and median prolactin levels in females from the high dose group were less than half of those of the combined control group, though the difference was not statistically significant due to the high variation of the values (high standard deviations) (**Table 30**).

Table 30. Serum prolactin values (µg/L) in females following 92 days of treatment[a]

ppm	0[b]	300 (20 mg/kg/day)	1000 (60 mg/kg/day) [c]	4000 (361 mg/kg/day) [c]
Day 92: mean	70.07 ± 174.51	237.53 ± 326.77	146.44 ± 181.97	**27.50 ± 29.83**
Day 92: median	26.43	55.52	88.79	**11.05**

[a] Data obtained from page 125 in the study report (MRID 49689128).
[b] Values from both controls groups were combined; N=20
[c] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)
* Statistically different from diestrus, p<0.05
** Statistically different from diestrus, p<0.01

In a third study, afidopyropen was administered to 20 female Fischer rats/group via the diet at dose levels of 0, 300, 1000, or 3000 ppm (0, 18, 81, or 368 mg/kg bw/day) [2] for 28 days (MRID 49689016). Additionally, 20 females serving as positive controls were administered the dopamine agonist bromocriptine (10 mg/kg bw/day) via gavage. To stimulate the release of endogenous prolactin from the pituitary, all animals received metoclopramide (500 µg/kg bw)

[2] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)

via i.p. injection on day 28.

In order to measure prolactin concentrations several approached were utilized. Measurements were taken at three fixed times in all animals regardless of the estrous cycle state of the rat including Study day -7, Study day 24 and study day 28. In addition to the samples taken at fixed times, from Day 0 to Day 24, prolactin measurements were taken only when an animal was in proestrus or estrus. Proestrus and estrus represent the highest plasma levels of prolactin, where treatment-related decreases in prolactin would most likely be detected. Immediately before sacrifice on Day 28, all animals were injected intraperitoneally with 500 µg/kg bw/d metoclopramide, a potent dopamine D2 antagonist, prior to prolactin measurement. Rat prolactin was measured with an ELISA kit (Demeditic, Germany). The prolactin assay was performed under internal laboratory quality control conditions with reference controls to assure reliable test results.

Blood samples on study days -7 and 24

In the pretest group (group 5), exposure to 500 µg metoclopramide/kg bw i.p produced the highest serum prolactin levels 0.5 hours after stimulation with decreasing values afterwards (data not shown). Therefore, blood samples on study day 28 in the main experiment were taken 0.5 hours after stimulation.

On day -7 (prior to compound administration), prolactin levels were found to be statistically significantly higher in proestrus and estrus, when compared to diestrus, confirming the expected results of cycle phases containing the highest prolactin concentrations (**Table 31**).

Table 31. Prolactin values prior to test compound administration (day -7) separated by sex cycle phase.

	Prolactin (ng/mL) Groups 0 - 4 Afidopyropen
Diestrus	Mean: 91.8 ± 259.9 (n=29) Median: 10.59
Proestrus	Mean: ****435.0 ± 299.0** (n=13) Median: **437.96**
Estrus	Mean: ****331.2 ± 411.6** (n=28) Median: **56.45**
Metestrus	Mean: 46.6 ± 165.1 (n=30) Median: 10.87

Data taken from page 76 of the study report (MRID 49689016).
* Statistically different from diestrus, p<0.05
** Statistically different from diestrus, p<0.01

On day 24, most of the control animals (group 0) were in metestrus (15/20) with the highest concentrations of prolactin found in proestrus and estrus animals (**Table 32**). A decrease in prolactin was observed in animals that were in metestrus from groups 2 and 3. This decrease was only found to be statistically significant in Group 2 animals when compared to day -7 control animals. In group 4, a decrease in prolactin was observed in animals that were in metestrus (when compared to day 24 control or day -7 control) and estrus (compared to day -7 control). Comparisons with day -7 control animals included all values with the exception of those belonging to the same animal, which was already used in the comparison group on study day 24. This was done to meet the assumption of independence of the measurements.

Table 32. Prolactin values (ng/mL) on day 24 separated by sex cycle phase.

	Group 0 Afidopyropen 0 ppm	Group 1 Afidopyropen 300 ppm (18 mg/kg/day)	Group 2 Afidopyropen 1000 ppm (81 mg/kg/day) [a]	Group 3 Afidopyropen 3000 ppm (368 mg/kg/day) [a]	Group 4 Bromocriptine (10 mg/kg bw/day)
	Females				
Diestrus	Mean: 53.17 ± 68.12 (n=2) Median: 53.17	Mean: 5.00 (n=1)	Mean: 25.30 ± 28.54 (n=4) Median: 13.13	Mean: 28.02 ± 27.34 (n=9) Median: 15.44	-
Proestrus	Mean: 757.35 ± 47.56 (n=2) Median: 757.35	Mean: 22.32 (n=1)	Mean: 640.51 ± 74.89 (n=3) Median: 627.07	-	-
Estrus	Mean: 764.29 (n=1)	Mean: 465.12 ± 650.05 (n=3) Median: 137.31	Mean: 792.20 ± 1091.64 (n=2) Median: 792.20	-	Mean: †**5.00 ± 0.00** (n=2) Median: **5.00**
Metestrus	Mean: 101.78 ± 234.94 (n=15) Median: 13.94	Mean: 187.24 ± 348.92 (n=15) Median: 13.39	Mean: †**28.23 ± 23.61** (n=11) Median: **19.50**	Mean: 25.82 ± 19.71 (n=11) Median: 22.15	Mean: ††****5.72 ± 1.96** (n=18) Median: **5.00**

Data taken from pages 77-83 of the study report (MRID 49689016).

[a] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)

* Statistically different from day 24 control, p<0.05

** Statistically different from day 24 control, p<0.01

† Statistically different from day -7 control, p<0.05, (with duplicates removed from the control group)

†† Statistically different from day -7 control, p<0.01, (with duplicates removed from the control group)

Blood samples in proestrus

Prolactin values in animals that were in proestrus were decreased (not statistically significant) in groups 2 and 3 on study days 0-4, and in group 3 on study days 15-22. Group 4 had a statistically significant decrease in prolactin values between days 0-3, and 16-22 (**Table 33**).

Table 33. Prolactin values (ng/mL) from proestrus.

	Group 0 Afidopyropen 0 ppm	Group 1 Afidopyropen 300 ppm (18 mg/kg/day)	Group 2 Afidopyropen 1000 ppm (81 mg/kg/day) [a]	Group 3 Afidopyropen 3000 ppm (368 mg/kg/day) [a]	Group 4 Bromocriptine (10 mg/kg bw/day)
	Females				
Day 0-4	Mean: 444.03 ± 343.06 (n=11) Median: 564.91	Mean: 395.71 ± 306.71 (n=10) Median: 438.12	Mean: 200.89 ± 223.03 (n=9) Median: 128.24	Mean: 167.67 ± 267.83 (n=9) Median: 27.84	
Day 0-3	Mean: 415.98 ± 370.48 (n=9) Median: 564.91				Mean: ****8.75 ± 6.88** (n=11) Median: **5.99**
Day 15-22	Mean: 640.80 ± 475.54 (n=7) Median: 864.84	Mean: 735.27 ± 167.57 (n=6) Median: 721.08	Mean: 604.07 ± 285.42 (n=9) Median: 484.64	Mean: 13.72 ± 9.86 (n=2) Median: 13.72	
Day 16-22	Mean: 746.77 ± 420.77 (n=6) Median: 880.36				Mean: ****8.49 ± 4.12** (n=13) Median: 6.03

Data taken from pages 84-87 (MRID 49689016).

[a] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)

* Statistically different from control, p<0.05

** Statistically different from control, p<0.01

Blood samples in estrus

Group 4 had a statistically significant decrease in prolactin values between days 18-22 (**Table 34**).

Table 34. Prolactin values (ng/mL) from estrus.

	Group 0 Afidopyropen 0 ppm	Group 1 Afidopyropen 300 ppm (18 mg/kg/day)	Group 2 Afidopyropen 1000 ppm (81 mg/kg/day) [a]	Group 3 Afidopyropen 3000 ppm (368 mg/kg/day) [a]	Group 4 Bromocriptine (10 mg/kg bw/day)
	Females				
Day18-22	Mean: 500.22 ± 491.09 (n=9) Median: 245.09	Mean: 578.95 ± 611.51 (n=10) Median: 415.32	Mean: 707.56 ± 421.79 (n=13) Median: 762.07		Mean: ****5.71 ± 1.07** (n=6) Median: **5.17**

Data taken from pages 88-89 (MRID 49689016).
[a] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01

Stimulation test with metoclopramide

On day 28, prior to stimulation using metoclopramide, levels of prolactin in group 4 in metestrus were statistically significantly elevated. This was also observed in group 4 in estrus, although due to the small number of animals in the control group, statistical significance could not be performed (**Table 35**).

Table 35. Prolactin values (ng/mL) on study day 28 prior to prolactin stimulation (basal values).

	Group 0 Afidopyropen 0 ppm	Group 1 Afidopyropen 300 ppm (18 mg/kg/day)	Group 2 Afidopyropen 1000 ppm (81 mg/kg/day) [a]	Group 3 Afidopyropen 3000 ppm (368 mg/kg/day) [a]	Group 4 Bromocriptine (10 mg/kg bw/day)
	Females				
Diestrus				Mean: 44.27 (n=1)	Mean: 34.69 (n=1)
Proestrus		Mean: 26.48 ± 6.34 (n=4) Median: 27.27	Mean: 41.95 ± 39.24 (n=2) Median: 41.95		Mean: 89.38 (n=1)
Estrus	Mean: 52.24 ± 27.92 (n=2) Median: 52.24	Mean: 70.98 ± 45.14 (n=5) Median: 62.48	Mean: 36.46 ± 30.17 (n=4) Median: 24.07		Mean: 107.42 ± 69.31 (n=7) Median: 104.41
Metestrus	Mean: 14.09 ± 7.82 (n=18) Median: 14.18	Mean: 32.86 ± 65.69 (n=11) Median: 13.54	Mean: 8.51 ± 4.85 (n=14) Median: 5.29	Mean: 11.38 ± 8.09 (n=19) Median: 8.85	Mean: ****96.31 ± 69.76** (n=11) Median: **95.73**

Data taken from pages 90-91 (MRID 49689016).
[a] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01

Half an hour after stimulation with metoclopramide, serum prolactin values in rats of test groups 2, 3 and 4 in metestrus were significantly lower compared to controls (**Table 36**).

Table 36. Prolactin values (ng/mL) on study day 28, 30 minutes after prolactin stimulation.

	Group 0 Afidopyropen 0 ppm	Group 1 Afidopyropen 300 ppm (18 mg/kg/day)	Group 2 Afidopyropen 1000 ppm (81 mg/kg/day) [a]	Group 3 Afidopyropen 3000 ppm (368 mg/kg/day) [a]	Group 4 Bromocriptine (10 mg/kg bw/day)
	Females				
Diestrus				Mean: 419.96 (n=1)	Mean: 153.13 (n=1)
Proestrus		Mean: 1153.00 ± 350.91 (n=4) Median: 1147.03	Mean: 1222.80 ± 179.04 (n=2) Median: 1222.80		Mean: 136.13 (n=1)
Estrus	Mean: 2659.85 ± 644.10 (n=2) Median: 2659.85	Mean: 2639.30 ± 432.05 (n=5) Median: 2530.50	Mean: 2353.68 ± 608.80 (n=4) Median: 2392.30		Mean: 375.06 ± 213.05 (n=7) Median: 400.58
Metestrus	Mean: 1063.47 ± 582.35 (n=18) Median: 919.94	Mean: 1298.10 ± 731.16 (n=11) Median: 1432.90	Mean: ***637.59 ± 190.91** (n=14) Median: **606.48**	Mean: ****313.33 ± 107.76** (n=19) Median: **314.43**	Mean: ****326.71 ± 170.90** (n=11) Median: **373.56**

Data taken from pages 92-93 (MRID 49689016).

[a] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)

* Statistically different from control, p<0.05

** Statistically different from control, p<0.01

The same statistical significances were obtained when for each individual the difference between the stimulated and the basal values at study day 28 was calculated (**Table 37**).

Table 37. Difference between prolactin (ng/mL) stimulated and basal values.

	Group 0 Afidopyropen 0 ppm	Group 1 Afidopyropen 300 ppm (18 mg/kg/day)	Group 2 Afidopyropen 1000 ppm (81 mg/kg/day) [a]	Group 3 Afidopyropen 3000 ppm (368 mg/kg/day) [a]	Group 4 Bromocriptine (10 mg/kg bw/day)
	Females				
Diestrus					Mean: 118.44 (n=1)
Proestrus					Mean: 47.18 (n=1)
Estrus	Mean: 2607.62 ± 672.03 (n=2) Median: 2607.62	Mean: 2568.32 ± 405.08 (n=5) Median: 2502.55	Mean: 2317.22 ± 612.26 (n=4) Median: 2336.92		Mean: 267.65 ± 166.8 (n=7) Median: 277.14
Metestrus	Mean: 1049.38 ± 580.56 (n=18) Median: 914.94	Mean: 1265.24 ± 717.06 (n=11) Median: 1419.36	Mean: ***629.08 ± 193.82** (n=14) Median: **594.96**	Mean: ****301.95 ± 102.31** (n=19) Median: **302.76**	Mean: ****230.4 ± 139.33** (n=11) Median: **208.15**

Data taken from pages 94-95 (MRID 49689016).

[a] The Agency notes that uterine tumors were determined to be treatment-related at 1000 ppm (50 mg/kg/day) and 3000 ppm (147 mg/kg/day)

* Statistically different from control, p<0.05

** Statistically different from control, p<0.01

CARC concluded that there is not sufficient evidence across all submitted studies to support a decrease in serum prolactin levels (Key Event #2). Changes in prolactin levels in the treated groups that were in proestrus and estrus (stages generally associated with the highest prolactin concentration) were not observed following stimulation with metoclopramide on day 28. A more pronounced decrease during these stages of the estrous cycle, and not just in the metestrus stage, would be expected if treatment with afidopyropen were to cause a decrease in prolactin levels. However, the CARC did note that there were very few animals in these stages of the estrous cycle on day 28 (n=2-4). In addition, even with the very limited evidence at the high dose for a decrease in serum prolactin, this dose level is higher (361 - 404 mg/kg/day)

than the tumorigenic dose level (50 mg/kg/day), and therefore, does not support this Key Event.

Data supporting Key Event #3 (Decreased corpus luteum support leading to decreased production of progesterone and a resulting estrogen dominance):

There is no specific study-related data to support this key event. However, the Registrant states that decreased corpus luteum support and the resulting decreased production in progesterone is a well-established consequence of decreased prolactin in the rat.

CARC concluded that there is no direct evidence or data from the Registrant submitted studies that afidopyropen decreases corpus luteum support leading to decreased production of progesterone and a resulting estrogen dominance (Key Event #3).

Data supporting Key Event #4 (Altered reproductive senescence in aged rats):

The Registrant states that there is a lack of precursor lesions to uterine adenocarcinoma (endometrial hyperproliferation) at early time points (up to and including the 1yr chronic rat study). If the mode of action were estrogen receptor agonism, proliferation of the mammary tissue and uterine tissue would be expected to be observed in early time points, subsequently progressing to uterine adenocarcinomas. However, in afidopyropen studies it was only in menopausal rats that altered proliferation of the uterus (increased) and mammary tissue (decreased) was observed. This E2: P imbalance only observed late in life indicates that the normal progesterone dominance that occurs in senescence and leads to a pseudopregnant state did not occur.

Additional evidence includes findings in the high dose 2-year rat cancer study (MRID 49688984). The non-neoplastic histopathology of the mammary gland reported a decreased incidence in the dilatation of the duct at both 1000 and 3000 ppm. The decreased incidence of this lesion was statistically significant at 3000 ppm (p<0.01) (**Table 38**).

Table 38: Incidence of selected histopathological findings in rat administered afidopyropen for 2-years (MRID 49688984)

Mammary gland	0	1000 (50 mg/kg/day)	3000 (147 mg/kg/day)
Dilation, duct +	22/50 (44%)	15/50 (30%)	11/50* (22%)
Dilation, duct ++	0/50	1/50 (2%)	0/50
Dilation, duct +++	1/50 (2%)	1/50 (2%)	0/50
Total (all severities) Dilation, duct	23/50 (46%)	17/50 (34%)	11/50 (22%)**

*: Significantly different (p<0.05) from 0 ppm group.
**: Significantly different (p<0.01) from 0 ppm group.

The Agency also notes the mammary gland histopathology findings in the low dose rat carcinogenicity study, specifically at 1000 ppm (MRID 49688983) (**Table 39**).

Table 39: Incidence of selected histopathological findings in rat administered afidopyropen for 2-years (MRID 49688983)

Mammary gland	0	100 (5 mg/kg/day)	300 (16 mg/kg/day)	1000 (51 mg/kg/day)
Dilation, duct +	9/50 (18%)	2/25 (8%)	2/19 (11%)	6/50 (12%)
Dilation, duct ++	1/50 (2%)	1/25 (4%)	1/19 (5%)	3/50 (6%)
Dilation, duct +++	1/50 (2%)	0/25	1/19 (5%)	1/50 (2%)
Total (all severities) Dilation, duct	11/50 (22%)	3/25 (12%)	4/19 (21%)	10/50 (20%)

The Registrant noted that the dilatation of the mammary gland duct is a normal consequence of reproductive senescence in the virgin female F344 rats as they become middle aged (from 8-14 months of age). As normal reproductive senescence ensues there is an increasing level of prolactin secretion that contributes to the development of a number of spontaneous morphologic changes that include increased secretions, duct dilation, alveolar and tubular epithelial hyperplasia, and periductal fibrosis. Xenobiotics that *increase* pituitary prolactin secretion like dopamine receptor antagonists can also cause these rat mammary gland changes. In contrast, dopamine agonists may *reduce* prolactin and decrease the incidence of the above histologic changes as well as the incidence of spontaneous mammary gland neoplasia[3]. The decreased incidence of mammary gland duct dilatation observed with afidopyropen treatment is consistent with the delayed entry into senescence, a known consequence of a decreased serum prolactin level.

CARC concluded that there was a decrease in mammary duct dilation at 3000 ppm; however, the decrease at 1000 ppm was modest in the high dose study and non-existent in the low dose study. In addition, when the results were combined from both studies, the dose response diminishes. These data do not adequately support altered reproductive senescence in aged rats (Key Event #4).

Data supporting Key Event #5 (Endometrial hyperproliferation):

There was no reported increase of hyperplasia in the uterus following afidopyropen exposure for 12 months (MRID 49688981 and MRID 49688982). In the low dose rat carcinogenicity study (MRID 49688983), slight endometrial hyperplasia was noted in control and treated animals; however, a dose-response was not observed and statistical significance was not reached at any dose level (**Table 40**).

Table 40. Summary of select non-neoplastic findings presented as number of animals with finding / number of animals examined.

ppm	0	100 (5 mg/kg/day)	300 (16 mg/kg/day)	1000 (51 mg/kg/day)
Females				
Uterus: slight endometrial hyperplasia – at necropsy	6 / 37 (16.2%)	7 / 38 (18.4%)	10 / 42 (23.8%)	9 / 40 (22.5%)
Uterus: slight endometrial hyperplasia – all animals	7 / 50 (14.0%)	8 / 50 (16.0%)	12 / 50 (24.0%)	9 / 50 (18.0%)

[3] Rudmann D., *et al*., Proliferative and nonproliferative lesions of the rat and mouse mammary, Zymbal's, preputial, and clitoral glands. Toxicol Pathol. 2012 Aug;40 (6 Suppl):7S-39S).

Data obtained from pages 113-157 in the study report (MRID 49688983)
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

In the high dose rat carcinogenicity study (MRID 49688984), a slight (not statistically significant) increase in uterine endometrial hyperplasia was observed at 1000 ppm with a statistically significant increase at 3000 ppm. It should be noted that the incidences in the control animals were comparable between the low and high dose carcinogenicity studies (**Table 41**).

Table 41. Summary of select non-neoplastic findings presented as number of animals with finding / number of animals examined.

ppm	0	1000 (50 mg/kg/day)	3000 (147 mg/kg/day)
Females			
Uterus - Hyperplasia, endometrium, slight – at necropsy	5 / 40 (13%)	9 / 40 (23%)	*11 / 32 (34%)
Uterus - Hyperplasia, endometrium, moderate – at necropsy	0 / 40	0 / 40	1 / 32 (3%)
Uterus - Hyperplasia, endometrium, severe – at necropsy	0 / 40	1 / 40 (3%)	1 / 32 (3%)
Uterus - Hyperplasia, endometrium, total – at necropsy	5 / 40 (13%)	10 / 40 (25%)	**13 / 32 (41%)
Uterus - Hyperplasia, endometrium, slight – all animals	7 / 50 (14%)	10 / 50 (20%)	13 / 50 (26%)
Uterus - Hyperplasia, endometrium, moderate – all animals	0 / 50	0 / 50	1 / 50 (2%)
Uterus - Hyperplasia, endometrium, severe – all animals	0 / 50	1 / 50 (2%)	2 / 50 (4%)
Uterus - Hyperplasia, endometrium, total – all animals	7 / 50 (14%)	11 / 50 (22%)	*16 / 50 (32%)

Data obtained from pages 113-150 in the study report (MRID 49688984)
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

CARC concluded that there is not sufficient evidence for an increase in endometrial hyperplasia (Key Event #5) at 1000 ppm when looked at across both the low dose and high dose rat carcinogenicity studies. There is evidence to support an increase in endometrial hyperplasia at 3000 ppm; however, when the data from both studies are combined, the dose response diminishes, and therefore, the CARC concluded there was only weak evidence of endometrial hyperplasia at 3000 ppm.

Data supporting <u>Key Event #6</u> (Promotion of uterine adenocarcinoma):

There is evidence for an increase in uterine adenocarcinomas in the rat at 1000 and 3000 ppm (**Table 6a**, **Table 14**, and **Table 22**).

The CARC concluded that there is evidence to support Key Event #6 and the uterine tumors were determined to be treatment-related at 1000 (50 mg/kg/day) and 3000 ppm (147 mg/kg/day).

<u>Overall conclusion</u>: Based on the lack of sufficient evidence to adequately support most of key events for a dopamine enhancement MOA for uterine tumors in female rats, the CARC concluded that the Registrant's postulated MOA for uterine tumors was not adequately demonstrated.

b. Dose-Response Relationship/Temporal Association

The Registrant has concluded that all of the key events associated with the formation of uterine adenocarcinomas occur at doses that exceed a kinetically derived maximum tolerated dose in the F344/DuCrlCrlj rat. This issue has previously been presented to the HED Toxicology Science Advisory Council (ToxSAC) and will be repeated here.

The Registrant claims that nonlinear kinetics, saturation of elimination, occurred at doses ≥15 mg/kg/bw. This argument is based on three main points.

1) The lack of saturation of absorption was demonstrated in two studies (MRID 49688953 and MRID 49688957) where the percent recovered AD did not change from high to low dose (e.g. 70.3% AD vs. 71.1% AD recovered after administration of 3 mg/kg and 300 mg/kg afidopyropen, respectively) (**Table 42**).

Table 42: Lack of saturation of absorption

MRID	Lab	Strain	% administered dose			
			Males		Females	
			3 mg/kg	300 mg/kg	3 mg/kg	300 mg/kg
49688953*	IET	Fischer	70.3	71.1	67.2	72.2
49688957**	BASF	Wistar	56.94	57.16	56.77	59.61

*Calculated as percent recovery from urine, bile and carcass
**Calculated as percent recovery from urine, bile, cage wash and carcass
Values shown are bioavailable (absorbed)

2) A change in the elimination pattern was also noted. Two studies (MRID 49688953 and MRID 49688957) exhibited, in females at the high dose, urinary excretion was increased, with biliary and fecal excretion decreased (**Table 43**). For male Fischer rats, bile excretion was decreased while urine excretion was increased from low to high dose. The registrant suggests that the altered excretion pattern at the high dose indications that saturation of excretion is occurring as the internal dose goes **supralinear**.

Table 43: Change in afidopyropen elimination pattern high vs. low dose

	% administered dose			
Sex (mg/kg)	Males (3)	Females (3)	Males (300)	Females (300)
MRID49688953 - Fischer, single dose				
Bile	53.10	53.28	40.16	40.45
Urine	16.23	**12.96**	29.91	**30.30**
Feces	22.37	26.73	26.36	24.05
MRID49688957 - Wistar, single dose				
Bile	39.16	45.49	41.30	36.16
Urine	17.43	**10.92**	15.18	**21.49**
Feces	30.01	31.68	31.97	22.65

To further support this argument, the registrant points to two studies (MRID 49688955 and MRID 49688953), where the metabolic pattern observed at the low dose is altered at the high

dose (**Figure 1**). At the high dose, metabolism of the parent to ME5343-T17 (M440017) is saturated, therefore, redirecting metabolism to a different pathway, resulting in a higher concentration of ME5343-T1 (M440I001) being excreted in the urine with a lower concentration of ME5343-T17 being excreted in the bile (**Table 44** and **Table 45**).

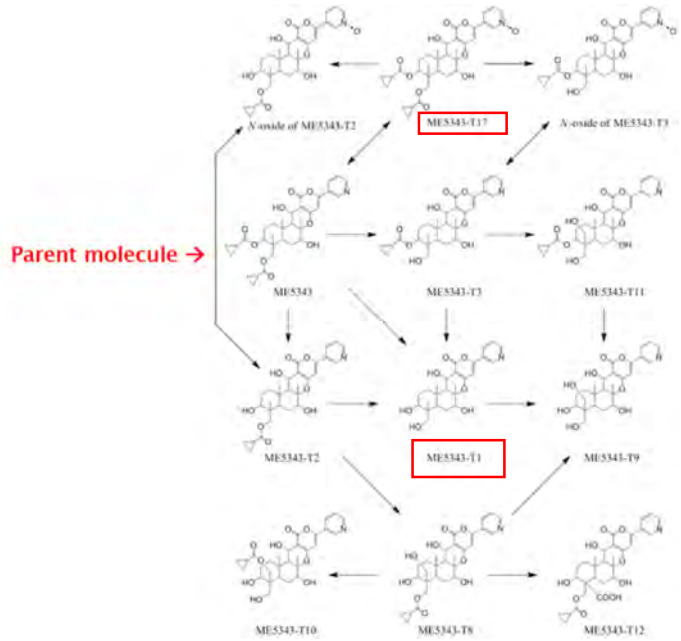


Figure 1: Change in afidopyropen elimination pattern

Table 44: Change in afidopyropen elimination pattern high vs. low dose

	% recovery of administered dose (MRID49688955) Wistar Rats, single dose			
Sex (mg/kg)	Males (3)	Females (3)	Males (300)	Females (300)
Urine				
M440I001	**2.18**	**1.89**	**10.55**	**10.14**
M440O17	0.93	0.44	0.37	0.72
Bile				
M440I001	3.59	2.25	3.46	3.71
M440O17	**20.52**	**28.52**	**10.43**	**8.28**

Table 45: Change in afidopyropen elimination pattern high vs. low dose

	% recovery of administered dose (MRID49688953) Fischer Rats, single dose			
Sex (mg/kg)	Males (3)	Females (3)	Males (300)	Females (300)
Urine				
M440I001	**2.47**	**1.96**	**9.64**	**8.17**
M440O17	1.46	0.62	0.55	0.59
Bile				
M440I001	10.0	6.19	13.6	11.72
M440O17	**20.30**	**31.78**	**4.95**	**6.35**

3) The final conclusion made by the registrant to support the claim of saturation of elimination was a disproportionate increase in plasma concentration (**Table 46** and **Table 47**).

Table 46: Disproportionate increase in plasma concentration; Female Wistar Rats (MRID 49688957); single dose

External Dose		Plasma Afidopyropen			
mg/kg bw/d	External Dose Difference	Cmax Ug-Eq/ml	Cmax Difference	AUC hr*ug-Eq/ml	AUC Difference
3	1X	0.40	1X	2.2	1X
30	10X	11.83	30X	543.9	247X
300	100X	61.11	153X	1003.3	456X

Compared to low dose:

- Mid dose: AUC value 25X higher than expected for linear kinetics.
- High dose: AUC value 5X higher than expected for linear kinetics

Compared to mid dose:

- High dose: a 10X increase in external dose only led to a 5X increase in Cmax or a 2X increase in AUC.

PMRA plotted the expected and observed AUC values (**Figure 2**). The mid-dose value is inconsistent with male Wistar data and female Fischer data. However, data **is not supralinear** as suggested by the registrant, but **sublinear**.

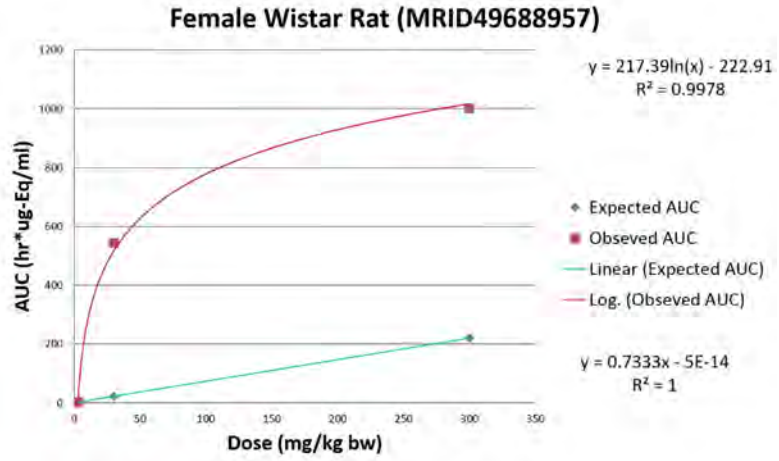


Figure 2: Expected vs. Observed AUC values

Table 47: Disproportionate increase in plasma concentration; Male Wistar Rats (MRID 49688957); single dose

External Dose		Plasma Afidopyropen			
mg/kg bw/d	External Dose Difference	Cmax Ug-Eq/ml	Cmax Difference	AUC hr*ug-Eq/ml	AUC Difference
3	1X	0.4	1X	2.1	1X
30	10X	6.24	16X	49.7	24X
300	100X	45.66	114X	783.6	373X

Compared to low dose:

- Mid dose: AUC value 2.4X higher than expected for linear kinetics.

- High dose: AUC value 3.7X higher than expected for linear kinetics
  Compared to mid dose:
- High dose: a 10X increase in external dose only led to a 16X in AUC.

PMRA plotted the expected and observed AUC values and demonstrated that the doses are actually rather linear (**Figure 3**).

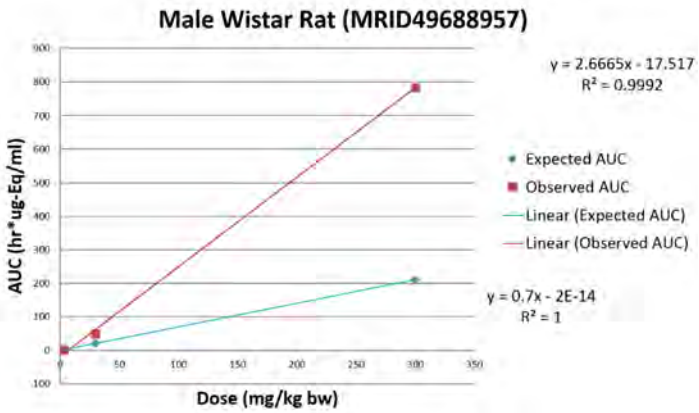


Figure 3: Expected vs. observed AUC

A similar pattern in AUC values was also demonstrated in several other studies:

- MRID 49689014: Female Fisher rats after 14 daily doses of non-radiolabeled afidopyropen followed by 1 dose of radiolabeled afidopyropen (**Table 48**).

Table 48: Disproportionate increase in plasma concentration; Female Fisher Rats

External Dose		Plasma Afidopyropen			
mg/kg bw/d	External Dose Difference	Cmax ug-Eq/ml	Cmax Difference	AUC hr*ug-Eq/ml	AUC Difference
Comparing to low dose:					
3	1X	0.262	1X	0.80	1X
15	5X	2.80	10.7X	10.8	13.5X
50	16.7X	6.98	26.7X	42.3	53X
Comparing mid to high dose:					
15	1X	2.80	1X	10.8	1X
50	3.3X	6.98	2.5X	42.3	3.9X

PMRA plotted the expected and observed AUC values and demonstrated that the doses are actually rather linear (**Figure 4**).

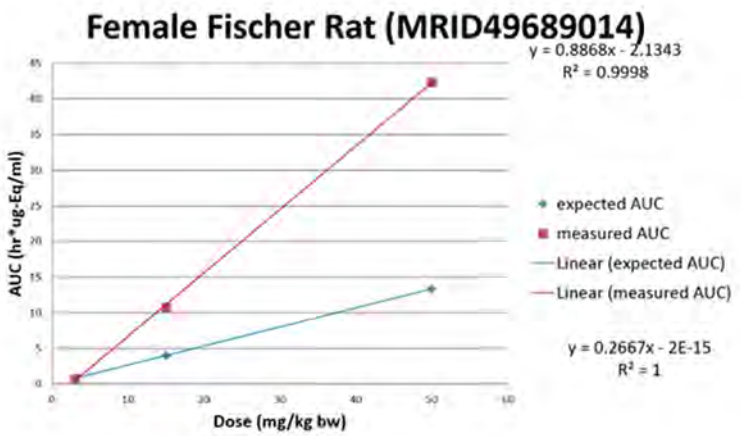


Figure 4: Expected vs. observed AUC

- MRID 49689014: Female Fischer rats after repeat dosing of 3, 15, or 50 mg/kg/bw. An unproportional increase in AUC value was demonstrated for parent, ME5343-T1, ME5343-T17, and CPCA-carnitine (**Table 49**). The magnitude of change was not as striking with metabolites as with parent compound.

Table 49: Disproportionate increase in plasma concentration; Female Fisher Rats

Analyte	External Dose		Plasma			
	mg/kg bw/d	External Dose Difference	Cmax ng-Eq/ml	Cmax Difference	AUD hr*ug-Eq/ml	AUD Difference
Afidopyropen	3	1X	24.7	1X	0.104	1X
	15	5X	1500	61X	4.53	44X
	50	17X	4750	192X	20.7	199X
M440I001	3	1X	-	-	0.725	1X
	15	5X	153	-	0.635	9X
	50	17X	457	-	2.69	37X
M440I0017	3	1X	3.91	1X	0.0782	1X
	15	5X	51.6	13X	0.482	6X
	50	17X	175	45X	1.35	17X
CPCA-carnitine M440I060	3	1X	195	1X	4.93	1X
	15	5X	1560	8X	36.2	7X
	50	17X	4190	22X	122.0	25X

- MRID 49689125: Male and female Fischer rats, single dose at 3 or 300 mg/kg bw, showed a similar disproportional increase in AUC values. The male AUC values were 5X higher than expected for linear kinetics and females 9X higher (data not shown).

Registrants Conclusions:

- Results indicate that nonlinear saturation kinetics occur for Wistar rats at doses greater than 30 mg/kg bw (**Figure 5**). The dose at which the inflection point for onset of

saturated PK is observed is well-separated from projected human doses resulting from compound use; therefore, the toxicological effects found only with **superlinear** kinetics are of questionable human relevance.

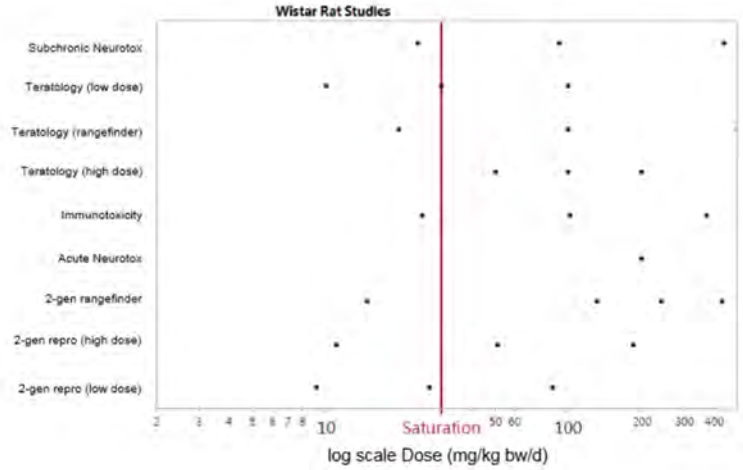


Figure 5: Plot of dose levels (log scale) used across afidopyropen data package with Wistar rats. The red line indicates the inflection point above which non-linear saturation kinetics occur (30 mg/kg/day).

- Results indicate that nonlinear saturation kinetics occur for Fischer rats dosed greater than 15 mg/kg bw (**Figure 6**).

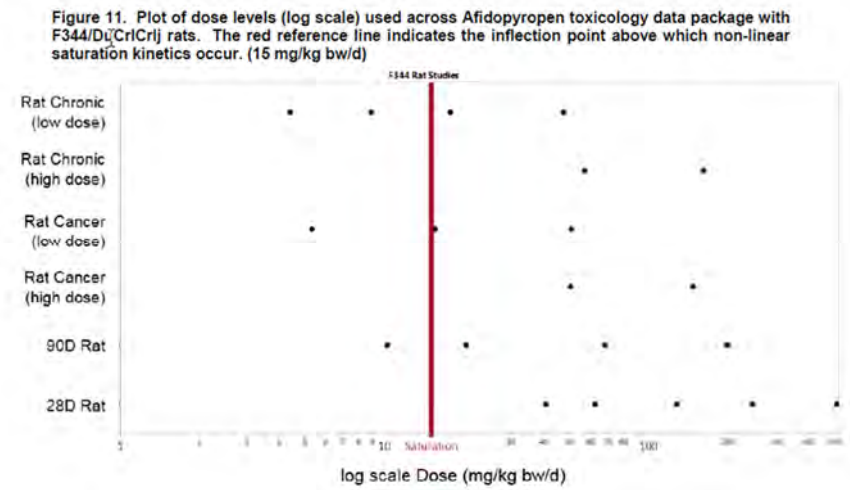


Figure 6: Plot of dose levels (log scale) used across afidopyropen data package with F344/DuCrlCrlj rats. The red reference line indicates the inflection point above which non-linear saturation kinetics occur (15 mg/kg/bw).

Taken from registrant submitted document (MRID 49689131):

"The Afidopyropen PK studies revealed that at high doses there was high oral absorption coupled with saturated saturation of elimination and metabolism. This saturation leads to a disproportionate increase in plasma concentration of Afidopyropen as doses increase.

Because the disproportionate increases in plasma concentrations occur at dose levels used in the Afidopyropen toxicology studies, the PK results are relevant to assess high-dose-specific toxicological effects observed with Afidopyropen.

The dose at which the inflection point for onset of saturated pharmacokinetics is observed is well separated from projected human doses resulting from Afidopyropen use. It is unlikely humans will be exposed to a dose level where ***superlinear*** *kinetics would occur. Toxicological effects observed with Afidopyropen that occur only at dose levels above a kinetically derived maximum tolerated dose (KMD) are of questionable human relevance."*

Other considerations from PMRA:

1) Calculation of the deviation from linearity (**Table 50**).

Table 50: AUC comparison mid to high dose

Dose mg/kg bw/d	External Dose Difference	AUC hr*ug-Eq/ml	AUC Difference	Deviation from linearity
Male Wistar Rat (MRID49688957)				
30	1X	49.7	1X	**1X**
300	10X	783.6	15.8X	**1.6X**
Female Wistar Rat (MRID49688957)				
30	1X	543.9	1X	**1X**
300	10X	1003.3	1.8X	**0.18X**
Female Fischer Rat (MRID49689014)				
15	1X	10.8	1X	**1X**
50	3.3X	42.3	3.9X	**1.2X**

Deviation = (AUC/AUC $_{\text{mid dose}}$) / (Dose/Mid dose)

2) Extrapolation of **supralinearity**; predict what point the high dose should have been if there was supralinearlity, using the equation of the curve or by multiplying by the dose increase and the low-mid dose supralinearlity (**Figure 7**).

Study	Mid dose AUC	Method	Extrapolated high dose AUC @ 50 mg/kg bw
		$y = 0.0378x^2 + 0.1533x + 3E\text{-}14$	102.165
Female Fischer	10.8 at 15 mg/kg bw	(High dose/mid dose)*(non linearity); (50/15)*2.7	97.2

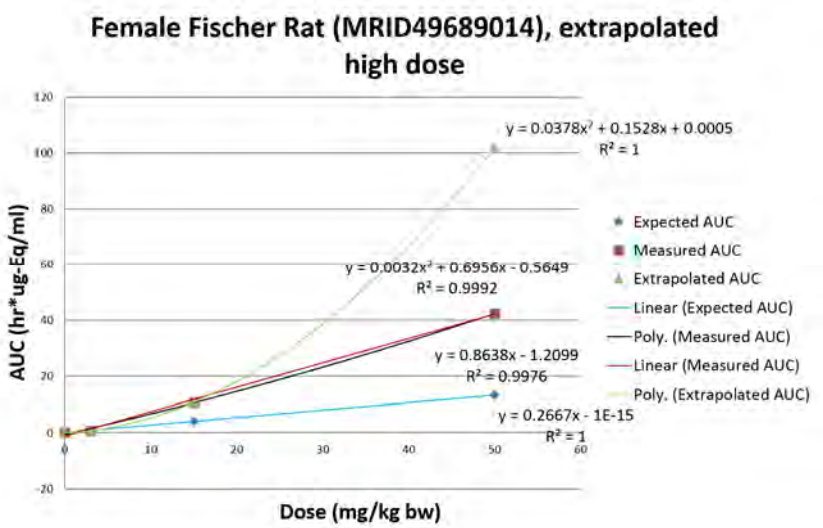


Figure 7: Extrapolation of supralinearlity using the equation of the curve

PMRA conclusion:

- Supralinearity was not demonstrated
  - Best fit for the data is linear
  - Some data (female Wistar) may be suspect
- There *may* be non-linearity in the bottom portion of the curve (i.e. two modes of excretion: active and/or passive transport), regardless, the inflection point was not determined with the available data.
- What they should have done: more doses than three, IV dose levels (to remove effect of absorption to focus on elimination)

Based on the points presented above, the ToxSAC concluded that saturation of elimination was not demonstrated and agreed with PMRAs conclusions. In order to properly demonstrate saturation of elimination additional information would be necessary, such as, time course data, more than three dose levels tested in the study, and conclusions on the relevance of these data in humans.

CARC agrees with PMRA and ToxSAC conclusions. Saturation of elimination has not been adequately demonstrated.

The Registrant concluded that there is a logical temporal relationship (**Table 51**) with the key events preceding the appearance of uterine adenocarcinomas. Dopamine agonism is expected to occur soon after afidopyropen exposure. Decreases in serum prolactin were observed within five days of the initiation of afidopyropen treatment. The lack of uterine proliferation at time points up to and including 1 year is consistent with this mode of action. Indications of delayed senescence are not observed until the end of the 2-year rat study, but this delay is consistent with this mechanism of action. It is not until the end of the two years of treatment that uterine hyperproliferation and adenocarcinoma are observed. This late development of hyperproliferation and adenocarcinoma is typical of the dopamine agonist mode of uterine adenocarcinoma formation.

Table 51: Dose-Response Temporality Concordance Table

Temporal →

Dose ↓

Dose (ppm)	Key Event 1 Dopamine Receptor Agonism	Key Event 2 Decreased serum prolactin levels	Key Event 3 E2:P Imbalance due to decreased CL support	Key Event 4 Altered reproductive senescence	Key Event 5 Endometrial hyper-proliferation	Key Event 6 Promotion of Uterine Tumors
100			-	-	-	-
300		-	-	-	-	-
1000	+*	+	+*	+	+	+
3000	+*	+	+*	+	+	+

+ indicates effect present; - indicates effect absent; Blank cell indicates no data.
*Indirect data based on earlier and later key events
CL: Corpus Luteum
----The dark line indicates the inflection point for observed saturation kinetics (15 mg/kg bw, approximately 300 ppm). Doses below this line exceed a kinetically derived maximum tolerated dose

CARC concluded that the Registrant proposed MOA for uterine tumors in female rats was not adequately demonstrated or supported. In turn, the dose-response relationship/temporal association was not relevant or demonstrated.

c. Biological Plausibility and Coherence (Abridged text below taken directly from registrant submitted proposed MOA; MRID 49689130)

The Registrant states that the observed D2 receptor functional agonism induced by afidopyropen can be attributed as a causal key event to produce the observed decrease in serum prolactin. Secondary to the direct bioassay confirming dopamine agonism, is the indirect evidence provided by decreased levels of serum prolactin at doses that caused increased uterine adenocarcinomas in rats. Both the direct D2 receptor bioassay, as well as the indirect measure of serum prolactin concentration in rat, are indicative that afidopyropen at high doses interacts with the D2 receptor. The effect on prolactin in the *in vivo* study confirms that the observed *in vitro* activation of the D2 receptor is not just a stand-alone finding only occurring *in vitro*, but that the same D2 activation occurs in the *in vivo* system and is biologically relevant, albeit at doses that are not human relevant.

The Registrant states that additional confidence that this [decreased prolactin-uterine adenocarcinoma] causal relationship is present at high doses of afidopyropen is provided by the characteristic lesion signature induced by hypoprolactinemia (both the increased incidence of uterine adenocarcinomas and reduced incidence of mammary neoplasia)[4] . Dopamine agonists do not always exhibit exactly the same lesion signature. However, even without establishing the initial key events for this MOA, afidopyropen would be suspected as a dopamine agonist, based only upon the profile of lesions observed in the cancer studies. The afidopyropen data supports a non-genotoxic, non-human relevant, threshold mode of action.

CARC concluded that the Registrant proposed MOA for uterine tumors in female rats was not adequately demonstrated or supported. In turn, the biological plausibility and coherence was not relevant.

d. Alternative Modes of Action

1. Alternate mode of action: The lesions are not treatment-related (Taken directly from registrant submitted proposed MOA; MRID 49689130)

The Registrant considered the possibility that the observed uterine adenocarcinomas may not be treatment-related. The low dose rat cancer study report came to this "not treatment-related" conclusion (the Agency notes this is the Registrant's conclusion) based upon the increased background incidence of this lesion in this F344/DuCrlCrlj strain of rat (MRID 49688983). Though there was no historical control available for this strain of rat at the facility conducting the study (Nisseiken Co Tokyo, Japan), a publication from Bozo Laboratories in Japan (2013) indicates a statistically significant rise in F344/DUCrlCrlj uterine adenocarcinomas in the past years, with a maximum incidence at 22% (**Table 52**)[5]. The Agency notes the low dose carcinogenicity study was performed between 2009-2011 while the high dose study between 2011-2013.

Table 52: **Incidence of Uterine Adenocarcinomas in F344/DUCrlCrlj rats at Bozo Laboratories.**

Years	1990-1999	2000-2004	2005-2009
No. Study	11	5	4
No. Rats	873	275	215
Mean (%)	3.3%	12.0%**	13.5%**
Range (%)	0-8%	9-16%	9-22%

** p<0.01 vs. 1990-1999 (Bonferroni t-test)

This increasing incidence of uterine tumors observed with the F344/DUCrlCrlj rats is in contrast to the incidence of uterine neoplasia observed in the F344/N strain used by the NTP, where

[4] Harleman JH., *et al*. A review of the incidence and coincidence of uterine and mammary tumors in Wistar and Sprague-Dawley rats based on the RITA database and the role of prolactin. Toxicol Pathol. 2012 Aug;40(6):926-3

[5] Kuroiwa, Y., *et al*. Transition of historical control data for high incidence tumors in f344 rats. J Toxicol Pathol. 2013 Jun;26(2):227-30.

incidence in a 2013 report was 0.29% for uterus adenoma and 0.29% for uterus carcinoma (*NTP Historical Controls report. All routes and vehicles. F344/N rats*. June 2013; 14 studies with start dates 2003-2005; Total N=700).

However, the Registrant concluded, that the circumstances surrounding the cancer studies indicate that these lesions are likely treatment-related given the historical and concurrent control data, the statistical significance presented, and the dose response for the tumor incidence.

2. **Alternate mode of action: Genotoxicity**

   Afidopyropen showed no evidence of causing genotoxicity (**Table 26**).

3. **Alternate Mode of Action: Endocrine (estrogen receptor agonist) mode of action (Abridged text below taken directly from registrant submitted proposed MOA; MRID 49689130)**

With uterine tumors, estrogen receptor agonism is an obvious potential mechanism for tumor formation. The estrogen to progesterone ratio (E2:P) is the critical endpoint for uterus tissue proliferation. Any direct alteration in effective E2 concentration would upset this critical ratio. A potential cause of perturbation of this ratio is E2 receptor agonism.

Figure 8: Key Events for hypothesized estradiol (E2) hormonal mediated formation of uterine adenocarcinomas

The Registrant stated that the afidopyropen data surrounding the rat reproduction studies as well as the lack of other endocrine disruption related endpoints do not indicate an estrogen receptor agonist. The rat 2-generation studies saw no consistent evidence of estrogenic activity. Other evidence against this mode of action includes the pattern of uterus tissue proliferation consistently observed in subchronic studies at high doses with the F344/DuCrlCrlj rat (not with other strains of rat or species). A significant reduction in uterus weight occurred as early as 14 days into treatment. Though this endpoint indicated that the uterus was a target in this strain of rat, this particular finding is not consistent with what would be expected with an estrogen receptor agonist where proliferation and weight increases would be observed. The uterus proliferation that was observed with afidopyropen treatment was only observed late in life - also not typical of an estrogen receptor agonist.

Though the Registrant stated that they saw no indication of estrogen receptor interaction in the guideline toxicology studies, several assays from the Endocrine Disruption Screening Program (EDSP) were conducted.

1. Estrogen receptor binding assay (OPPTS 890.1250): Assay on Afidopyropen and two major rat metabolites (M440I001 and M440I002) (MRID 49689010)

2. Transcriptional activation assay (OPPTS 890.1300): Afidopyropen; (MRID 49689009)

Results from the three independent estrogen receptor binding assays showed M440I001 and M440I002 to be "non-interacting" with the estrogen receptor. Afidopyropen was classified of "equivocal." A result of "equivocal" is not a positive or a potential positive classification, but rather, it means the result is ambiguous, or cannot be properly identified due to limitations of the assay (MRID 49689010).

In two independent runs of the transcriptional activation assay, afidopyropen did not increase luciferase activity at any of the viable concentrations tested (RPC_{max}<10%). These data suggest afidopyropen is not an agonist of human estrogen receptor alpha (hERα) in the HeLa-9903 model system (MRID 49689009).

The Registrant states that the data do not support the first key event in this estrogen receptor agonist hypothesized mode of action. Though there are shared key events with the dopamine agonist MOA, the timing of the key events observed in the toxicology studies is not consistent with an estrogen receptor agonist mode of action.

4. Alternate Mode of Action: Enzyme induction (Taken from registrant submitted proposed MOA; MRID 49689130)

Estradiol (E2) mediated carcinogenesis may also have a non-hormonal source.

Figure 9: Hypothesized MOA for Non-hormonal estradiol (E2) mediated carcinogenesis

One of the known modes of action for formation of uterine adenocarcinomas is enzyme induction. The metabolism of estradiol to 4-hydroxyestradiol - a suspected tumor initiator and/or promoter in estrogen responsive tissues such as the uterus is primarily driven by CYP1B1.

Key events for this hypothesized MOA are as follows:

1. Enzymes responsible for estradiol (E2) metabolism are induced (e.g. CYP1B1, CYP1A1)
2. Unbalanced metabolism of E2 *via* CYP1B1 leads to an increased production of genotoxic metabolite 4OH-E2.
3. Formation of 4OH-E2 DNA adducts
4. Error prone base excision / repair
5. Abnormal uterine cell proliferation
6. Uterine Adenocarcinomas

The Registrant states that there was suggestive evidence for this hypothesized mode of action from the guideline toxicology studies. The evidence consisted of treatment-related liver effects.

In both the 28-day (MRID 49688964) and the 90-day (MRID 49688967) rat feeding studies, the absolute and relative liver weights of female rats were significantly increased, compared to the controls, at dietary concentrations of 1000-3000 ppm. In addition, histopathology in the 90-day study revealed vacuolar changes in 8/10 and 10/10 females at dietary concentrations of 1000 or 3000 ppm (MRID 49688967). Microscopic pathology was not performed in the 28-day rat study. These observations raised the possibility of enzyme induction, which formed the basis for further investigation of this endpoint.

To investigate the possibility of afidopyropen enzyme induction, an *in vivo* enzyme induction assay was conducted (MRID 49689008). The primary goal was to investigate the first hypothesized key event for this potential mode of action, the induction of CYP1B1. Female Fischer 344 rats were treated for 14 days with 3000 ppm (197 mg/kg bw/day) afidopyropen. At termination, the liver was processed for isolation of microsomes and the analysis of CYP1A1 and CYP1B1 enzymatic activity. Liver and uteri were processed for Taqman® analysis of CYP1A1 and CYP1B1 mRNA expression.

In the treated rats, there was a slight yet statistically significant 1.6-fold increase in hepatic microsomal ethoxyresorufin-O-deethylation (EROD) (used as a marker for CYP1A) at day 14 following administration of afidopyropen at 3000 ppm in the diet. Estradiol-2- hydroxylation was similarly increased 1.7-fold. The increases in hepatic microsomal enzyme activities were accompanied by a statistically significant 4-fold increase in hepatic CYP1A1 mRNA. There was a slight increase in hepatic CYP1B1 expression, but it was not statistically significant. Uterine CYP1A1 mRNA was significantly increased 57-fold in afidopyropen-treated rats. However, there were no changes in the expression of uterine CYP1B1.

In addition, the Registrant stated that the elevated CYP1A1 mRNA levels as well the slightly increased EROD activity are not representative for a prototypical Aryl-Hydrocarbon Receptor (AHR)-inducer (EROD induction >100 fold (e.g. NTP reports 520, 521, 525, 529, 525, 530, 531) mRNA >1000 fold).

The Registrant concluded that because the crucial key event (enzyme induction) for this mode of action was not observed, this MOA was not considered plausible for the induction of uterine adenocarcinomas in rat.

5. Additional data for consideration by the Agency

In addition, the Agency notes the atrophy and estrous cycle findings that were observed in several of the Registrant submitted studies. In the 28-day study with afidopyropen, ovary, uterine, cervix, and vagina atrophy was noted in all high dose animals (**Tables 53**; MRID 49689016). In addition, the high dose animals also experienced a decrease in absolute and relative uterine (↓59-63%) and ovary (↓43-46%) weight.

Table 53. Summary of female microscopic findings (MRID 49689016)

	Group 0 Afidopyropen 0 ppm n = 20	Group 1 Afidopyropen 300 ppm (18 mg/kg bw/d) n = 20	Group 2 Afidopyropen 1000 ppm (81 mg/kg bw/d) n = 20	Group 3 Afidopyropen 3000 ppm (368 mg/kg bw/d) n = 20
	Females			
Ovaries: Atrophy, diffuse	1	0	2	20
Uterus: Atrophy, diffuse	0	0	0	20
Cervix: Atrophy, diffuse	0	0	0	20
Vagina: Atrophy, diffuse	1	0	2	20
Vagina: mucification increased	8	5	11	20

There was a statistically significantly decreased number of estrous cycles in animals of the high dose group. The cycle length in animals of the high dose group was also increased (**Table 54**). Only 3/20 females had a second proestrus or estrous cycle detected within the observation period. The cycle length was greater than 24 days for 17/20 animals in the high dose animals. The actual cycle length could not be determined since it extended passed the end of the study.

A statistically significantly increased number of cycles and statistically significantly decreased cycle length was observed for the animals in the bromocriptine positive control group.

Table 54. Summary of estrous cycle findings (MRID 49689016)

	Group 0 Afidopyropen 0 ppm n = 20	Group 1 Afidopyropen 300 ppm (18 mg/kg bw/d) n = 20	Group 2 Afidopyropen 1000 ppm (81 mg/kg bw/d) n = 20	Group 3 Afidopyropen 3000 ppm (368 mg/kg bw/d) n = 20	Group 4 Bromocriptine 10 mg/kg bw/d n = 20
	Females				
No. cycles	3.70 ± 1.13	3.50 ± 1.36	3.35 ± 0.93	**0.15 ± 0.37	**5.05 ± 0.89
Cycle length (days)	6.79 ± 2.49	8.27 ± 4.22	6.97 ± 2.60	[a]8.00 ± 10.39 (n=3)	**4.85 ± 0.73

Data taken from page 73 of the study report.
[a] in all other animals the cycle length was prolonged to at least 24 days.
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01

Uterine and cervix atrophy were also noted in several 90-day rat studies with afidopyropen. In the first 90-day study, uterus atrophy was noted in 6/10 high dose females and cervix atrophy in 4/10 high dose females (**Table 55**; MRID 49689127). Also noted in this study was a decrease in absolute and relative ovarian (↓29%) and uterine (↓50-51%) weight. There were no treatment-related findings observed in the number of cycles or cycle lengths for females of any dose group.

Table 55. Incidence of select histopathological findings following 90 days of treatment (N =10) (MRID 49689127)

	0 ppm	4000 ppm (404 mg/kg bw/d)
	Females	
Uterus: Atrophy, diffuse	0	6

Cervix: **Atrophy**	0	4

[a] Data obtained from pages 135-136 in the study report
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

In the second 90-day rat study, uterine and cervix atrophy were noted in 9/10 animals in the high dose group (**Table 56**). Also noted was a decrease in absolute and relative ovarian (↓32-37%) and uterine (↓51-54%) weight.

Table 56. Incidence of select histopathological findings following 90 days of treatment (N =10) (MRID 49689128)

	0 ppm	**4000 ppm (360 mg/kg bw/d)**
	Females	
Uterus: **Atrophy, diffuse**	-	9
Cervix: **Atrophy, diffuse**	-	9

[a] Data obtained from pages 138-139 in the study report
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

In the second 90-day rat study, there were no statistically significant differences observed in the number of cycles or cycle length for females in any dose group (**Table 57**). However, at the high dose, the number of cycles was lower and the cycle length was longer in comparison to controls.

Table 57. Estrous cycle in females (n=10) (MRID 49689128)

	0	**300 ppm (20 mg/kg bw/d)**	**1000 ppm (60 mg/kg bw/d)**	**4000 ppm (360 mg/kg bw/d)**
Number of cycles	3.90 ± 0.99	4.00 ± 0.82	3.80 ± 0.79	3.00 ± 1.05
Cycle length (days)	4.15 ± 0.82	4.30 ± 0.64	4.60 ± 0.83	5.58 ± 1.72

In the third 90-day rat study, atrophy was noted in 1/10 high dose females; however, the food consumption of the 4000 ppm group was 197 mg/kg bw/day, while the previously discussed studies had nearly double the food consumption on a mg/kg bw/day scale (**Table 58**). Also noted was a decrease in absolute and relative ovarian (↓12-18%) and uterine (↓11-16%) weight.

Table 58. Incidence of select histopathological findings following 90 days of treatment (N =10) (MRID 49689126)

	0 ppm	**4000 ppm (197 mg/kg bw/d)**
	Females	
Uterus: **Atrophy, diffuse**	0	1
Cystic dilation, glands	0	1

[a] Data obtained from pages 131-132 in the study report

In the third 90-day rat study, there were no statistically significant differences observed in the number of cycles or cycle length for females in any dose group (**Table 59**). However, at the high dose, the number of cycles was lower and the cycle length was very slightly longer in comparison to controls.

Table 59. Estrous cycle in females (n=10) (MRID 49689126)

	0	300 ppm (26 mg/kg bw/d)	1000 ppm (98 mg/kg bw/d)	4000 ppm (197 mg/kg bw/d)
Number of cycles	5.00 ± 0.47	4.30 ± 0.82	4.40 ± 0.70	4.20 ± 1.69
Cycle length (days)	3.81 ± 0.17	4.25 ± 0.86	4.01 ± 0.25	3.95 ± 0.54

The CARC concluded that these data support altered reproductive cyclicity. The change in cyclicity is not stated as part of the Registrant proposed MOA and could potentially influence an alternative MOA that was not considered by the Registrant.

e. Uncertainties, Inconsistencies, and Data Gaps (Taken directly from registrant submitted proposed MOA; MRID 49689130)

The Registrant stated that based upon reported data from other laboratories in Japan, the F344/DUCrlCrlj strain of rat appears to be uniquely susceptible to uterine adenocarcinomas. The lack of historical control data in the laboratory conducting the rat cancer studies (Nisseiken - Tokyo, Japan) make evaluation of the lesion incidence difficult. With a more robust historical data, the uterine adenocarcinoma incidence observed with afidopyropen may have fallen within historical control.

This tumor type only was observed with the F344/DuCrlCrlj rat, a strain that has shown a high and increasing background incidence of this lesion. This mechanism is theoretically available to the mouse, but no afidopyropen-induced increase in uterine adenocarcinomas was observed with the mouse. Dopamine agonist-induced uterine carcinomas have not been reported in literature with the mouse. It may be the F344/DuCrlCrlj rat is uniquely susceptible rodent / rodent strain to the dopamine agonist effect provided by afidopyropen treatment. Alternatively, mouse PK (not studied) may not exhibit dose-dependent saturation of clearance of afidopyropen or its other dopamine-agonist active metabolites as has been experimentally demonstrated in rats.

CARC concluded that the Registrant proposed MOA for uterine tumors in female rats was not adequately demonstrated or supported. In turn, the uncertainties, inconsistencies and data gaps noted were not relevant.

f. Human Relevance (Taken directly from registrant submitted proposed MOA; MRID 49689130)

1. *Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?*

The Registrant concluded that there is strong evidence to support the dopamine agonism in F344/DuCrlCrlj rats, resulting decreases in serum prolactin, and the associated delayed senescence caused by estrogen dominance and the ultimate uterine adenocarcinomas.

2. *Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?*

The Registrant concluded that there is a difference in the role of prolactin between rat and human in female reproductive cyclicity. Tumorigenic effects on the uterus by compounds that increase dopamine levels are considered to be rat specific and not relevant to pathophysiological conditions in the human, based on qualitative species differences between rat and human[6]. This difference is further supported through epidemiological studies with compounds like bromocriptine (a potent dopamine agonist). Bromocriptine induces uterine adenocarcinomas in rats. However, in women, clinical studies show no effect of bromocriptine on follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone levels or endometrial histology[7].

3. *Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?*

The registrant concluded that this document has presented evidence that shows the described MOA for afidopyropen occurs only at doses at least three times higher than the inflection point where saturation of excretion occurs and the internal dose becomes supra-linear. Where the dose is proportional to exposure, there is no indication of any of the key events in the described mode of action. Therefore, this is a mode of action that is only present at doses with clear saturation kinetics and represents a high dose restricted mode of action in rats.

Human exposure will be greater than 4000X the dose where saturation kinetics occurred in the rat. This was calculated using the endpoint, 8 mg/kg bw from the 1yr dog study, leading to an expected ADI of 0.08 mg/kg bw/day. The current Registrant submitted risk assessment shows expected afidopyropen human dietary exposure to reach a maximum of 0.002704 mg/kg bw. Therefore, there is little likelihood that humans will be exposed to dose levels where saturation kinetics (and the associated high-dose restricted mode of action) occur. It is not known if afidopyropen metabolism in rat's parallels that in humans. However, the extremely low estimates of human exposure indicate that excretion and metabolism of afidopyropen would be very unlikely to be saturated in humans, and thus not able to create metabolic conditions responsible for blood levels of afidopyropen and its metabolites seen at high, saturated doses in rats.

CARC concluded that the Registrant proposed MOA for uterine tumors in female rats was not adequately demonstrated or supported. In turn, the human relevance noted was not relevant.

[6] Brott DA, *et al.* A peripherally restricted P2Y12 receptor antagonist altered rat tumor incidences with no human relevance: Mode of action consistent with dopamine agonism. Toxicol Rep. 2014 Nov 20; 1:1202-1212.

[7] Toxicologic Pathology. Volume 22, Number 2, 1994.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT OF THE EVIDENCE

The following represents CARC's overall assessment of the data presented at the October 18, 2017 and November 1, 2017 meetings for afidopyropen:

Rats

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the adrenal tumors in males did not display a dose response, were not statistically significant (Fisher's Exact Test or Exact Trend Test), and there were no corresponding non-neoplastic lesions at any dose level. **The CARC concluded that the adrenal tumors were not treatment-related at any dose tested.**

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the combined lung tumors (adenomas and carcinomas) in males did not reach statistical significance (Fisher's Exact Test or Exact Trend Test), and no corresponding non-neoplastic lesions were noted at any dose level. **The CARC concluded that the lung tumors were not treatment-related at any dose tested.**

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the benign liver tumors (adenomas) in males did reach statistical significance ($p \leq 0.05$ for both Fisher's Exact Test and Exact Trend Test) and was outside of the historical control range and above the historical control mean at 3000 ppm. However, there was a weak dose response, no malignant tumors were observed (carcinomas), and the only noteworthy non-neoplastic finding was altered foci (predominantly slight in degree). **The CARC concluded that the liver adenomas were treatment-related at 3000 ppm**.

- When tumor data were combined from the low and high dose rat carcinogenicity studies, the uterine tumors in females displayed a dose response, reached statistical significance at 1000 and 3000 ppm ($p \leq 0.01$ for both Fisher's Exact Test and Exact Trend Test) for both adenocarcinomas and combined adenocarcinomas and adenomas, were outside the historical control range (at 3000 ppm), were above the historical control mean (at 1000 and 3000 ppm), and displayed uterine hyperplasia (increased incidence at 1000 and 3000 ppm). **The CARC concluded that the uterine tumors were treatment-related at 1000 and 3000 ppm.**

- The CARC concluded that dosing was adequate and not excessive for the combined low and high dose carcinogenicity studies in the rat.

Mice

- There were no treatment-related tumors in the mouse carcinogenicity study.

- Dosing was considered adequate and not excessive in the mouse carcinogenicity study based on a decrease in absolute body weight in females, clinical signs, increases in hematological blood parameters, spleen and ovary weights, an increase in pale colored liver.

Mutagenicity

- There is no concern for mutagenicity as determined from a battery of genotoxicity assays.

Structural Activity Relationships

- There are no structurally similar chemicals to inform structural activity relationships (SAR) for afidopyropen.

Registrant's Proposed MOA for Uterine Tumors in Rats

The Registrant proposed a dopamine enhancement mode of action via agonism of the dopamine receptor, leading to decreased serum prolactin levels, and through a cascade of key events, uterine tumors**. The CARC concluded that the submitted data do not adequately demonstrate the proposed MOA based on the following considerations:**

- Key Event #1: The CARC concluded that there is no direct evidence from the Registrant submitted studies that afidopyropen is an agonist of the dopamine receptor. Even though multiple *in silico* and *in vitro* binding assay results were presented, the overall weight-of-evidence conclusion still did not support agonism of the dopamine receptor for either parent or any metabolites tested.

- Key Event #2: The CARC concluded that there is not sufficient evidence across all submitted studies to support a decrease in serum prolactin levels. There is limited evidence of lower prolactin concentrations at the high dose in one study, but this effect was not consistently observed or present to the same extent as positive control chemical (bromocriptine) and was only reported at a higher dose level (361 - 404 mg/kg/day) than the tumorigenic dose level (50 mg/kg/day). In addition, prolactin concentrations were *higher* in the 92-day study at 1000 ppm, where a tumorigenic effect was seen in the carcinogenicity study. The only evidence of decreased prolactin at 1000 ppm was limited to the "metestrus group" on day 24, but such a stage-specific effects would potentially be confounded by altered cyclicity (noted in other studies).

- Key Event #3: The CARC concluded that there is no direct evidence or data from the Registrant submitted studies that afidopyropen decreases corpus luteum support leading to decreased production of progesterone and a resulting estrogen dominance.

- Key Event #4: The CARC concluded that there is evidence of altered cyclicity, but the effects occur inconsistently across studies; do not correspond well with those of

bromocriptine, the positive control agent; and do not support delayed senescence at the tumorigenic dose (1000 ppm). There was a decrease in mammary duct dilation at 3000 ppm; however, (a) no mammary gland effects were seen at 12 months in the high-dose study or 24 months at the tumorigenic dose (1000 ppm) in either the low-dose or high-dose rat studies; (b) when the results were combined from both studies, the dose response diminishes; and (c) this effect is not necessarily specific to altered reproductive senescence in aged rats.

- Key Event #5: The CARC concluded that there is not sufficient evidence for an increase in endometrial hyperplasia at 1000 ppm when looked at across both the low dose and high dose rat carcinogenicity studies. There is evidence to support an increase in endometrial hyperplasia at the 3000 ppm dose at 24 months. When the data from both studies are combined, the dose response diminishes, and there was no increase in endometrial hyperplasia seen at 12 months in the high-dose study. The CARC concluded there was weak evidence of endometrial hyperplasia at 3000 ppm.

- Key Event #6*:* The CARC concluded that there is evidence to support the formation of uterine tumors and determined the tumors to be treatment-related at 1000 and 3000 ppm.

Conclusion: Based on the deficiencies identified above, there is insufficient evidence to support the proposed MOA for rat uterine tumors induced by afidopyropen.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March 2005), the CARC classified afidopyropen as "***Suggestive Evidence of Carcinogenic Potential***" based on uterine tumors in female rats. There is insufficient evidence to support the proposed uterine tumor MOA in female rats. There was no treatment related tumors observed in the mouse carcinogenicity study and no concern for mutagenicity.

The *Final Guidelines for Carcinogen Risk Assessment* (March, 2005) were consulted regarding the benign liver tumors in male rats. The *Guidelines* state:

- *"Benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis. There is a range of possibilities for their overall significance."*
- *"[O]bservation of a benign tumor response alone may have no significant health hazard implications when other sources of evidence show no suggestion of carcinogenicity."*
- *"[I]n assessing findings from animal studies, a greater proportion of malignancy is weighed more heavily than is a response with a greater proportion of benign tumors."*

In the case of afidopyropen, the incidence of liver adenomas was not significantly increased in either the low dose or high dose study alone; a statistically significant increase was observed at the highest dose tested (3000 ppm) only when the studies were combined. These tumors did not progress to malignancy in either the low or high dose rat carcinogenicity studies and were not considered treatment-related in the mouse carcinogenicity study (due to the small magnitude of malignant neoplasms and the relative comparison to historical controls). The only noteworthy non-neoplastic finding was altered cell foci of the liver. Neither hypertrophy or hyperplasia were noted in the low dose or high dose rat carcinogenicity study. While the CARC did consider the liver adenomas to be treatment-related, based on the weight-of-evidence and guidance noted above, the benign tumors were not considered a concern in the overall cancer classification. The classification of "*Suggestive Evidence of Carcinogenic Potential*" is based on the uterine tumors in female rats (one sex/one species).

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification of human cancer risk is not required. The chronic Reference Dose (RfD) will adequately account for all chronic toxicity, including carcinogenicity, which could result from exposure to afidopyropen.

VIII. BIBLIOGRAPHY

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